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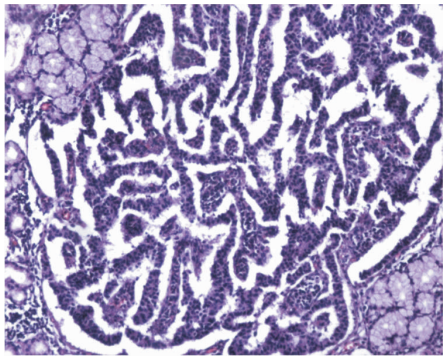

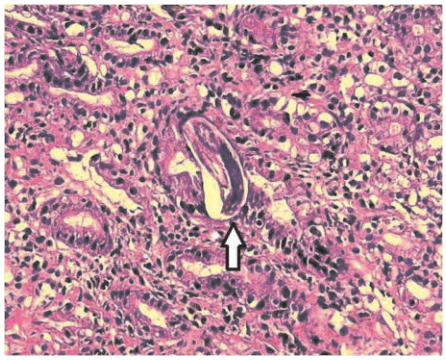

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SRI RAMACHANDRA JOUNRAL OF MEDICINE
JAN-JUNE 2013
CONTENT

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From the Editor's Desk

I am happy that the Jan-June 2013 issue of the SRJM is being released. This is the first issue after the new Editorial Board took charge.

The issue has three Original Articles, one Review Article, three Case Reports and one Image in Medicine. The standard of articles is gradually improving and I take this opportunity to place on record the tireless services rendered by the Joint Editors, the Editorial Board Members, Peer Reviewers, Authors, Printer and the back office staff members.

We plan to bring the July-Dec 2013 issue by end of April and Jan-June 2014 issue by end of June 2014.

Withing you all happy reading.

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STUDY OF TIME KINETICS OF MONOCLONAL ANTIBODIES TO *CHLAMYDIA TRACHOMATIS* TO DEFINE AN OPTIMUM TITER

Amrita Vats[a], Aruna Mittal[a]

ABSTRACT

Background and Objective : *Monoclonal based diagnostic assay for Chlamydia trachomatis was developed in our lab and initial screening was done to identify wells containing hybridoma that secrete antibody of desired specificity. In this study we have tried to define an optimum titer as a functional dilution or working concentration of an antibody samples to achieve a minimum level of acceptable value for specific detection for an assay method.*

Material and Methods *: Hybridoma tested complete Growth media (CG Media) containing RPMI 1640, 10% FBS(foetal bovine serum was used for revival of clones. The hybrid clones were expanded for production of anti-chlamydial monoclonal antibodies in complete growth medium initially in 24 well plates and expanded into culture flasks and were regularly observed till obtained 90% confluency. Subsequently supernatants were collected from cultured hybrid clones at day 3 and day 5 and were checked for secreting antibody to C.trachomatis by ELISA.*

Results : *Of the three hybrid clones, clone F10.5 had a titer of 1:8000 in supernatant collected from day 3 and day 5, however when supernatants were pooled from day 3 and 5, the titer was 1:10000 Further in clone B3.3 the titer was 1:8000 on day 3 and on subsequent pooling the titer was 1:4000.Similarly for clone E4.2, the antibody titer was 1:4000 at day 3 and 5, subsequent to pooling, the titer was 1:8000 .The best optical density(OD) values in all the three clones was detected at dilution of 1:1000.*

Conclusion : *In this study we observed that all the three clones mentioned (F10.5, B3.3, and E4.2) showed best reactivity at 1:1000 dilution and high OD is achievable at day 3.Clone F10.5 had a titer of 1: 10000 upon pooling, while for clone B3.3 and clone E4.2 the titer was 1:4000 and 1:8000 respectively. Thus it can be interpreted that a given antibody (clone), titer correlates mainly to concentration. .*

Key words: *Antibody titer, ELISA, Monoclonal antibodies, PNT baseline, Serial dilution.*

SRJM 2013;6:1-6

INTRODUCTION

Genital *Chlamydia trachomatis* infections are the most prevalent sexually transmitted bacterial disease recognized throughout the world. In majority of the women, long-term persistent *C. trachomatis* infection are often asymptomatic or clinically silent .[1] Chronic chlamydial infection may affect female upper genital tract leading to secondary tubal infertility, pelvic inflammatory disease (PID), chronic pelvic pain, salpingitis and ectopic pregnancies. [2] A World Health Organization (WHO) study documented that 18-20% of infertile women are infected with *C. trachomatis* worldwide [3] and in India, 28-30% of infertile women were reported to be *C. trachomatis* infected, which is quite high in terms of world scenario.[4] Diagnosis of *C.trachomatis* is important for proper treatment and prevention of chlamydial infection. We developed monoclonal antibody (Mab) based diagnostic assay for *C.trachomatis* infections. [5]

Monoclonal antibody is obtained from a single clone of hybridoma cell line and is known for their specificities to a single epitope of complex antigen. Pre-fusion parameters are largely optimized but several post fusion parameters are important for stabilizing hybridomas to expanded culture. [6] Freshly isolated hybrid cell cultures grow slowly and the volume of cell culture is expanded either *in vivo* (in animals) or *in vitro* (in culture flasks). Once the monoclonality of the cultures has been established, the clones are grown sequentially in increasing volumes of culture medium to develop a suitable stock of antibody producing cells.

The type of assay used to detect the secreting antibody depends on the nature of the antigen and the type of antibody desired. Technically simple, sensitive and convenient assay such as ELISA are used to screen large number of supernatants to identify the wells containing desired antibody. Once a clone has shown to secrete antigen specific Mab, efforts should be taken to preserve this clone, allowing them to grow to confluency and supernatants should be screened periodically for antibody activity.

The purpose of screening is to identify wells containing hybridomas that secrete antibody of desired specificity. The initial screening for antibody activity should be done as soon as growth of hybrid cells is seen under the microscope or upon a change in culture medium pH. In this study, we describe the expansion of hybridoma culture and screening for secreting antibody as a steady source of monoclonal antibodies (Mabs). ELISA was used for screening the supernatants for secreting antibody and to study the time kinetics. Here we have tried to define an optimum titer as a functional dilution (or working concentration of an antibody samples) to achieve a minimum level of acceptable value or specific detection for an assay method. The optimum antibody concentration is determined for each hybridoma clone by using a series of dilutions to interpret the best detectable level antibody titer

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MATERIALS AND METHODS

Cell Culture for revival of clones: The methodology used were as described earlier.[5] Hybridoma Tested RPMI 1640 (Roswell park memorial institute) (Himedia, India) containing sodium bi carbonate was used for revival of clones. Complete Growth media (CG Media) containing RPMI 1640, 10% FBS(foetal bovine serum) (PAA Laboratories, Austria), 200mM Glutamine (Sigma,USA), and Gentamicin (Himedia, India) 10 μg/ml as antibacterial agent and Amphotericin B (Himedia, India) 2 μg/ml as antifungal. This complete media was filter sterilized.

One vial of each cryopreserved hybridoma clone (anti *C trachomatis* monoclonal antibody) namely E 4.2, B3.3, F 10.5 clones were taken from liquid nitrogen container, quickly thawed in water bath at 37°C and subsequently transferred in to falcon tube containing 5 ml of RPMI 1640 without serum. This cell suspension centrifuged at 1000 rpm for 5 minutes at 4°C, supernatant discarded and pellet re-suspended in complete growth media . Viability for clone E4.2 was >90%, for clone B3.3 was >95% and for clone F10.5 was >98%.These cells were seeded separately initially in 24 well plate (seeding density 2 x 105) containing 1 ml of complete growth media in each well.

Expansion of hybrid clones: The anti-chlamydial monoclonal antibody hybrid clones were expanded in complete growth medium initially in 24 well plates (Greiner, Germany), were regularly observed till 90% confluency was obtained. After 48 hours of full confluency, clones were passaged by gentle shaking so that these were detached from the bottom and pipetted out gently. This cell suspension was again transferred into the falcon tube containing CG media, after centrifugation pellet re-suspend in the CG media and cells were seeded in to 6 well plates (Greiner, Germany) with 1 X 10^6 cells per ml. The clones obtained full confluency after 72 hours. These confluent cells were further expanded and passaged in CG medium at 2 x 10^6 Cells per ml in 25 cm^2 tissue culture flasks (Greiner, Germany) and further to 75 cm^2 at 37° C in 5 % CO_2.

Cryopreservation of hybrid clones: Hybrid cells were stored in cryovials in liquid nitrogen. Briefly Cells were centrifuged and re-suspended in freezing media containing 90% heat inactivated FBS and 10 % Dimethyl sulfoxide (DMSO Sigma-Aldrich, USA) at a concentration of 2 X 10^6 cells per ml . The suspension was distributed in 1 ml aliquots in to fresh and sterile cryovials (Greiner, Germany). The cryovials were tightly closed and placed in cryobox's subsequently kept at 4° C for 2 hours, -20° C for 4 hours and -70° C for overnight. Finally cryovials with cells were transferred in to liquid nitrogen for prolong preservation.

ELISA for checking secreted antibody in cultured hybrid clones (Reactivity in supernatants) : Supernatants were collected from cultured hybrid clones at day3 and day5 and were checked for secreting antibody by ELISA. Polysterene high binding 96 well ELISA plates (Greiner,Germany) were coated with supernatants (secreted antibody) 50 μl each with neat, 1:10,1:50,1:100,1:500, 1:1000 and 1: 2000, 1:4000, 1:8000 and 1:10000 diluted monoclonal antibodies and incubated overnight at 4°C. After incubation the plates were washed with PBS containing 0.05% tween 20 (Hi-Media,India) 3 to 4 times .2% BSA (SRL, India) 50μl in each well was then added and incubated for 2 hrs. Subsequently plates were washed 3 to 4 times with PBS and 0.05% tween 20 to remove excess BSA. Added 50μl of antigen/peptide (*C.trachomatis*) to the wells. One well was kept without antigen as negative control containing only PBS.Plates were incubated for 1 hr at 37°C and washed 3-4 times with PBS tween 20. Added 50 μl of secondary Rabbit anti mouse IgG HRP peroxidase conjugated antibody (dilution1:40000,Sigma,St louis,USA) in each well and incubated for 1 hr at 37°C and again washed 3 to 4 times with PBS tween 20. Added 50μl of TMB substrate (Merck Bangalore Genei) and incubated for 10 to15 min at room temperature for colour development .Subsequently the reaction was stopped by adding 50μl of stop solution IN H2SO4.Opical density reading was taken in ELISA reader(Labsystem, Multiskan, Germany) at 450nm.

Statistical analysis: Student t test was used to determine the p value between different antibody dilution and at day 3, 5, and pooled. Mean absorbance of negative control plus three standard deviation units (M+3 SD) were calculated at each dilution for these supernatants.[7] The resulting three standard deviation baseline unit values were then plotted against each dilution. M+3 SD indicates positive and negative threshold (PNT) baseline .The observed end point titer for assay was highest supernatant dilution that yielded an optical density (OD) greater than the value that defined the cutoff between negative and positive results.

RESULTS

Morphology of clones in culture as seen under phase contrast microscope: The hybrid clones appeared to be small rounded in morphology with clear cell boundaries. These hybrid clones were first feebly attached to the bottom of the wells. There was no contamination observed (Fig1).

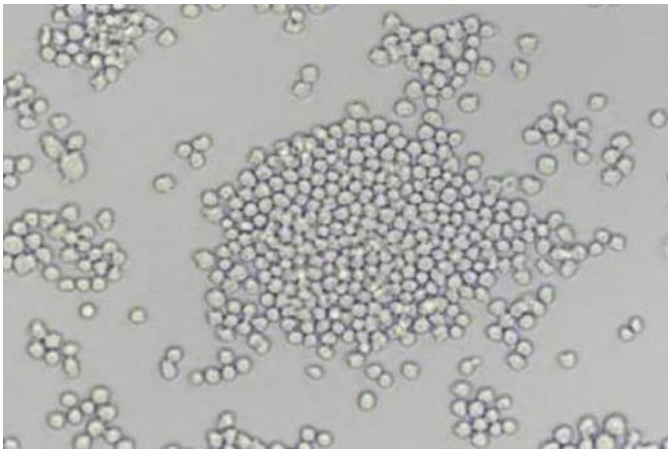

Fig-1: Microphotograph showing hybridoma clone in culture

ELISA results for checking reactivity in cultured hybridoma supernatants from the three clones : To check the reactivity of the three clones, supernatants collected from day 3, and 5 were initially diluted to 1:10, 1:50, and 1:100. It was observed that in clone F10.5 and Clone B3.3 showed a high OD value in 1:50 and 1:100 which is highly significant to 1:10 dilution ($p < ***0.05$). In clone E4.2 there was similar reactivity and significant OD values ($p = 0.05$). Further the three clones were subjected to check the reactivity from 1:500 to 1:10,000 and subsequent to pooling. (Fig 2 and Table1) Of the three hybrid clones, clone F10.5 showed a titer of 1:8,000 in supernatant collected from day 3 and day 5.When supernatant were pooled from day 3 and 5 and checked for the reactivity ,the titer was 1:10,000 with ($P > 0.05$) which was significant compare to day 3 or day 5. On days 3 and day 5 there was not much significant difference in reactivity. The maximum OD values was on 1:1000 dilutions which was highly significant to 1:4000 ($< *0.05$), 1:8000 ($p = 0.05$). In clone B3.3, the antibody titer was 1:8000 at day 3. At day 5 and subsequent to pooling the titer was 1:4000. The best OD values was again on 1:1000 having a significance level of ($p \geq 0.05$) in response to 1:2000 , 1:4000, 1:8000,1:10000 dilution of supernatant. In pooled supernatant the reactivity was in close range and significant compare to OD values of day 3 and day 5 ($p > 0.05$). There was not much difference in reactivity level of supernatant from day 3 day 5. Clone E4.2 showed a titer of 1:4000 at day 3 and day 5. However, when supernatant were pooled, they showed a titer level of 1:8000. The maximum OD value was achievable at 1:500 and 1:1000 dilutions which was significantly higher to ($p \leq ***0.05$) to dilution 1:2000, 1:4000, 1:8000 and 1:10000. In clone E4.2 there was similar gradation of reactivity level at day 3, 5 and upon pooling (Fig 2, Table 1).

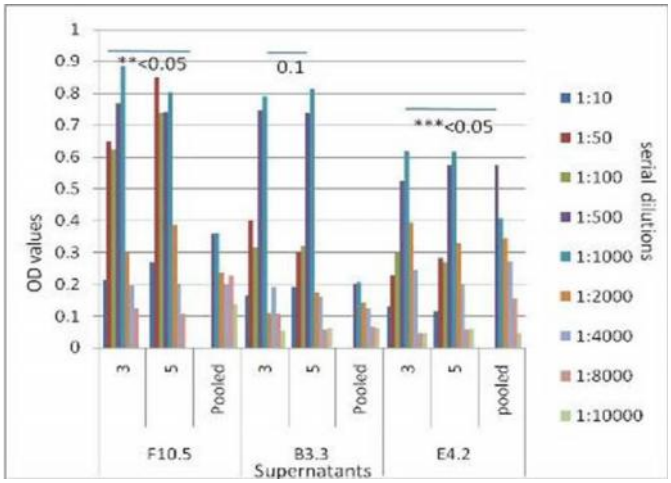

Fig-2: Screening of Hybridoma supernatants by ELISA . Absorbance value (OD values) are represented on Y axis. X axis denotes supernatants collected at day 3,5 and after pooling for the three clones (F10.5, B3.3, & E4.2).

Table: 1 ELISA OD values obtained by screening the hybridoma supernatants using specific antigen (C.trachomatis L2 peptide)

Clone from day	Supernatants	1:10	1:50	1:100	1:500	1:1000	1:2000	1:4000	1:8000	1:10000
F10.5	3	0.214 (0.081)	0.648 (0.091)	0.624 (0.120)	0.768 (0.103)	0.883 (0.103)	0.297 (0.064)	0.195 (0.087)	0.125 (0.079)	0.095 (0.060)
	5	0.267 (0.078)	0.847 (0.091)	0.738 (0.205)	0.741 (0.103)	0.801 (0.114)	0.384 (0.096)	0.197 (0.099)	0.106 (0.078)	0.089 (0.08)
	Pooled	**ND**	**ND**	**ND**	**0.357 (0.076)**	**0.36 (0.074)**	**0.236 (0.087)**	**0.201 (0.085)**	**0.225 (0.079)**	**0.136 (0.060)**
B3.3	**3**	**0.163 (0.095)**	**0.401 (0.138)**	**0.315 (0.120)**	**0.745 (0.255)**	**0.788 (0.119)**	**(0.147) (0.087)**	**0.191 (0.091)**	**0.106 (0.064)**	**0.054 (0.06)**
	5	**0.191 (0.078)**	**0.3 (0.121)**	**0.321 (0.143)**	**0.736 (0.259)**	**0.814 (0.115)**	**0.173 (0.062)**	**0.159 (0.096)**	**0.057 (0.65)**	**0.062 (0.079)**
	Pooled	**ND**	**ND**	**ND**	**0.2 (0.067)**	**0.205 (0.067)**	**0.14 (0.069)**	**0.125 (0.078)**	**0.064 (0.058)**	**0.062 (0.06)**
E4.2	**3**	**0.13 (0.022)**	**0.228 (0.064)**	**0.299 (0.09)**	**0.522 (0.108)**	**0.619 (0.080)**	**0.394 (0.093)**	**0.243 (0.074)**	**0.047 (0.059)**	**0.046 (0.056)**
	5	**0.115 (0.052)**	**0.283 (0.074)**	**0.268 (0.085)**	**0.528 (0.091)**	**0.616 (0.076)**	**0.328 (0.080)**	**0.200 (0.067)**	**0.056 (0.050)**	**0.059 (0.079)**
	Pooled	**ND**	**ND**	**ND**	**0.571 (0.052)**	**0.406 (0.091)**	**0.345 (0.082)**	**0.271 (0.093)**	**0.154 (0.056)**	**0.045 (0.056)**

*ND: not done

* Values in parenthesis indicating in Blue colour are corresponding negative control value

Standard serial dilution method of determining observed ELISA antibody titers with PNT baseline : To find the endpoint titer, mean of the negative control from each dilutions (starting from1:10 to 1:10000) accumulating supernatant from day 3, day 5 and pooled were calculated. The mean OD ± 1 standard deviation (SD) for each dilutions were also calculated .The mean OD ± 1 standard deviation (SD) at 1:1000 dilution are as follows:

- for clone F10.5 it is 0.097 ± 0.157,
- for clone B3.3 it is 0.10333 ± 0.18 and
- for clone E4.2 is 0.082 ± 0.103.(table 2).

The observed titer was determined as the dilution, at which the supernatant absorbance crosses the PNT line and can be depicted as the predictive value for a positive result (table 2 and fig 3,) For clone F10.5 the highest threshold is 0.3 OD at a dilution of 1:100, for clone B3.3 it is 0.5 at a dilution of 1:500, for clone E4.2 it is 0.2 at 1:100 dilution (Fig 3).

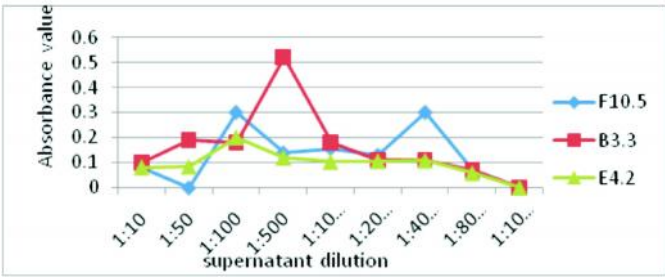

Fig-3: PNT baseline M + 3SD indicating positive and negative threshold .Y-axis denotes absorbance value(OD values), X-axis denotes supernatant dilution (1:10, 1:50, 1:100, 1:500, 1:1000, 1:2000, 1:4000, 1:8000,1:10,000).

Relationship between antibody dilution and OD values expressed as a hyperbolic curve : The highest peak of OD values of clone F10.5 (0.624-0.883; 0.847-0.801) were in the similar range from 1:50 to 1:1000 dilution and from 1:2000 to 1:8000 the OD values (0.297 0.125;0.384-0.106)

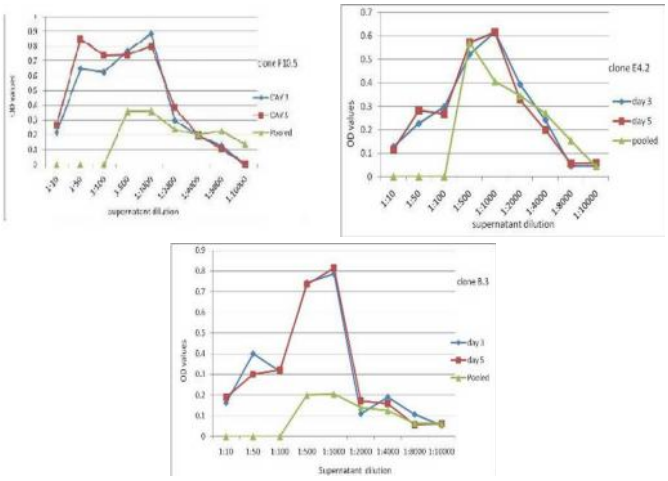

Fig: 4. Standard graphs to show effect of dilution on antibody and antigen reaction system. Y axis denotes OD values, X –axis denotes supernatant dilution.

gradually declined, however were significantly higher than the control value.(Fig 4) .While clone B3.3 express the greatest dilution of 1:8000 with high OD values in the range of (0.401-0.788) from 1:50 to 1: 1000 and gradual decrease in OD values (0.147 0.106) from 1:2000 -1:8000. While E4.2, the highest dilution of reactivity .Similarly for E4.2, the highest peak of OD values is 0.6 with gradual decline from 1:2000. (Fig 4).

Table 2 : Standard serial dilution method of determining observed ELISA antibody titers with PNT baseline.

Dilution F10.5	Mean	SD	M + 3SD
1:10	0.0795	0.00212	0.08
1:50	0.091	0	
1:100	0.1625	0.060	0.3
1:500	0.094	0.015	0.139
1:1000	0.097	0.020	0.157
1:2000	0.082	0.016	0.13
1:4000	0.09033	0.075	0.3
1:8000	0.078	0.0005	0.07
1:10000	0.06		
B3.3	**Mean**	**SD**	**M + 3SD**
1:10	0.0865	0.012	0.1
1:50	0.1625	0.0120	0.19
1:100	0.1315	0.0162	0.18
1:500	0.193667	0.1097	0.522
1:1000	0.100333	0.028	0.18
1:2000	0.072667	0.0128	0.111
1:4000	0.08833	0.009	0.11
1:8000	0.062333	0.003	0.07133
1:10000	0.06633	0 .01	0.09
E4.2	**Mean**	**SD**	**M + 3SD**
1:10	0.037	0.015	0.082
1:50	0.069	0.005	0.084
1:100	0.0875	0.002	0.2
1:500	0.0995	0.007	0.120
1:1000	0.082333	0.007	0.103
1:2000	0.085	0.007	0.106
1:4000	0.078	0.01	0.108
1:8000	0.055	0.001	0.058
1:10000	0.063667		

DISCUSSION

High prevalence of genital *C.trachomatis* infection has been reported in symptomatic patients in India and there is need to develop an indigenous monoclonal antibody based

diagnostic assay which is cost effective and can be used for reliable diagnosis of *C.trachomatis* infection.

Monoclonal antibodies (Mabs) have become indispensable for finer *"in vitro"* applications as well as in increasing usage in human diagnostics and therapeutics. Major challenges to ensure a stable, healthy hybridoma culture as a source of monoclonal antibodies, the time and cost involved in such production and also the isotype of the Mabs produced. The IgG isotype antibodies are preferable to the initial IgM response as for variety of applications owing to the high affinity. The hybridomas are fragile initially after fusion and are different from parental cell types. Several factors such as random chromosomal loss can either turn secretory cells non-secretory or the hybridoma might even collapse and fail to expand.

Quantitaive screening of antibodies secreted by hybridomas provides useful information in the selection of optimal clones particularly where high capacity production is important. Knowledge of antibody concentration is important not only in the ranking and selection of clones but also in designing functional assays and monitoring production levels of clones at different time intervals. The development of Monoclonal antibodies to *C.trachomatis* has already made a remarkable impact on the diagnosis of chlamydial infection.[8-9]

In our study, the screening was done using technique relatively higher sensitivity (that did not require concentration of supernatants) such as ELISA which also avoids the subjective bias and laborious microscopic examination required for the fluorescence test antigen.

ELISA with antimouse IgG confirmed the IgG isotype of the secreted Mabs and ELISA with the antigen (*C.trachomatis*) gave us the specific nature of the antibodies. As there are not many papers relating to the time kinetics of hybridoma clones to establish the antibody titer of secreting antibody, hence we studied the titer of developed monoclonal antibody (*C.trachomatis*) in secreting hybidoma culture supernatants for reactivity in all the above mentioned three clones above mentioned , at different time interval i.e. at day 3 and day 5 by ELISA. We observed that all the three clones mentioned (F10.5, B3.3, and E4.2) showed best reactivity at 1:1000 dilutions and high OD is achievable at day 3 and were significantly higher than the control value. It is earlier reported that titer of an antibody is also dependent on concentration of epitope [10] and it may be that there is a constant epitope concentration at 1:50 to 1:1000 dilution of clone F10.5 and we can see little differences from one dilution to the next unless there is a prozone effect.[11]. Clone F10.5 on pooling showed an antibody titer of 1:10000 which was significantly higher than the control value, indicating that intrinsic affinity of clone F10.5 is high. While clone B3.3 express the greatest dilution of 1:8000 at day 3. At day 5 and upon pooling the titer were 1:4000 with significantly higher OD values of (0.3-0.814) to control. The OD values were in the similar range upon pooling (0.2-0.125). For clone E4.2, the highest dilution of reactivity was 1:14000 at day 3 and day 5 , and upon pooling, the titer level was 1:8000 with maximum OD value at 1:500 and 1:1000 dilutions which was highly significant ($p \leq ***0.05$) at dilution 1:2000, 1:4000, 1:8000 and 1:10000. So it can be interpreted that a given antibody (clone), titer correlates to concentration. Here, Clone F10.5 and E4.2 are secretory for high affinity antibody, therefore it reacts with the antigen and gave more intense color than clone B3.3 and, which may be low affinity antibody. The other possibility is that the antibody in clone B3.3 had become inactivated (denatured, degraded). The rate of binding between antibody and antigen is dependent on the affinity constant which in turn can be affected by temperature, pH and solvent composition which also control the extent of antibody and antigen complex. [12]

Further occurrence of co-operativity (lateral Fc interaction and binding site heterogeneity), steric hindrance conditions such as epitope density and especially frequency of bivalent binding; under such conditions the interaction between antigen and antibody does not obey the law of mass action ; may be some of these factors responsible for low reactivity of clone B3.3.

Dilution based quantitative assay based on the linear relationship between ELISA OD values and optimum dilution have been reported for several organisms, sera etc.[13-14] The application of this type of quantitative serial dilution assay offers considerable scope for testing of large number of samples more practical. We therefore decided to investigate the suitability of serial dilution of supernatant obtained from hybridoma clone to detect the optimal end point titer which involves a linear relationship between ELISA OD values at that dilution were the ELISA end point titer was strongest. At a certain higher dilution of antibody i.e 1:2000 onwards , the plateau of the signal started to decline significantly, halfway down to the minimum signal i.e at the point of steepest decline of the slope (Fig 4). The relationship between the antibody dilution and OD values is expressed as a hyperbolic curve and only in certain ranges it can be approximated as a straight line/linear, hence it might be possible that these clones showing different shapes of dilution curve and that the shape of hyperbolic curve could be changed by a number of factors (e.g., time of incubation, type of antigen). [15] In case of endpoint titer method, result may change depending on the threshold of optical density (e.g the data set using 3SD above the highest OD may be quite different from the data using 0.6 for E4.2, 0.8 for B3.3, 0.9 for F10.5 as threshold) because curve of the clone are not parallel and it is difficult to judge which threshold is better.

ACKNOWLEDGMENTS

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PREVALENCE OF OBESITY AND ITS ASSOCIATED FACTORS IN AN URBAN AREA OF ANDHRA PRADESH

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ABSTRACT

Background *: Obesity is seen as the most important, fast growing and emerging health problem worldwide affecting children and adults alike as a result of improper dietary pattern and adoption of unhealthy lifestyles. Obesity is now rising in the Indian population with increase in prevalence in southern states like in Andhra Pradesh.*

Objectives : *To study the prevalence of obesity, its associated factors among the adult population (20-60 years) in the field practice area of Urban Health Centre, Saraswathi Nagar, Nellore.*

Materials and Methods : *A descriptive cross-sectional study of the sample population of 550 study subjects in the age group of 20-60 years were selected by Systematic Random Sampling technique. Data was collected by interviewing the study subjects using a pre-designed, pretested structured questionnaire. All the data was analyzed by using SPSS version 17.0 for windows.*

Results : *The prevalence of obesity among the study subjects was 53.1%, 49.5% in males and 50.5% in females. Prevalence of Abdominal obesity using waist circumference was 68.4% and using waist-hip ratio was 52.5%.Prevalence of obesity was high (61.5%) in 41-50 years age group. The association of obesity with age, community, occupation, nature of work, type of family, socio-economic class, family H/o obesity, intake of energy rich foods, emotional disturbances, H/o alcohol intake and smoking was found to be statistically significant. Hypertension and Diabetes had a significant association with obesity.*

Conclusion : *The study shows a high prevalence of obesity among the urban population (20-60 years) of Nellore city. Public health interventions like encouraging physical activity by environmental changes that increase or maintain incidental daily activity and has leisure pursuits and promotion of healthy diet that is low in fat, high in complex carbohydrates and containing large amount of fresh fruits and vegetables.*

Key words : *Obesity, waist circumference, waist-hip ratio, prevalence.*

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INTRODUCTION

Throughout most of the human history, weight gain and fat storage have been viewed as signs of health and prosperity. Today, however as standards of living continue to rise, weight gain and obesity are posing a growing threat to the health of the people in countries all over the world.[1]

Globally in 2005 approximately 1.6 billion adults (15+ ages) worldwide were overweight and 400 million adults of them were obese. By 2015 approximately 2.3 billion adults will be overweight and >700 million will be obese.[2]

The prevalence of obesity in South-East Asia region according to WHO studies conducted in 1991 showed that 15.2% in men and 23.2% in women were having BMI 25-29.99 and 3% of men and 3.8% of women had a BMI $\geq$30.[1] In the women, overweight and obesity problems were more serious in the Indian population; 17.1% of Indian women had a BMI over 30 compared to 8.8% of Malay and 4.3% of Chinese women.[1] Indian overweight population was about 70 million as on 15th July 2009. In the past 20 yrs the prevalence of obesity is tripled in developing countries that have been adopting a western life style involving decreased physical activity and over consumption of cheap, energy dense food.[2]

Available data on prevalence of obesity from different published studies; from different states of India suggest that the prevalence ranged from 10-50 %.[3,12] The prevalence of obesity is maximum in Punjab i.e. 30.3% in males and in females in 37.5%. The prevalence of obesity in Andhra Pradesh is, 17.6% among males and 22.7% among females. The various studies on obesity[12] in India is shown in Table - 1.

Very few community based studies conducted on obesity in Andhra Pradesh are available in various scientific journals and also no such study was done on obesity in Nellore city.

Taking the above facts into consideration, a community based study on obesity was undertaken to estimate the prevalence, the risk factors and its complications among the adult population (20-60 years) residing in the field practice area of Urban Health Centre, Saraswathi Nagar under Narayana Medical College, Nellore.

MATERIALS AND METHODS

The present cross sectional community based study was conducted in the field practice area of Urban Health Centre of Narayana Medical College, Nellore, Andhra Pradesh located at Saraswathi Nagar. NFHS-3 published data suggest that the prevalence of obesity in urban population in males is 22.2% and in females is 28.9%.[3] Based on the above, the prevalence of obesity in adults was taken as 25% on average. Sample size was estimated at 5% level of significance with an allowable error of 15%.

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Table 1: The prevalence of obesity in different parts of India

Author	City/Centre	Year	Prevalence of Obesity(%) Male	Female
Dhurandhar & Kulkarni	Bombay	1992	10.1 - 53.1	-
Gopinath et al	Delhi	1994	21.3	33.4
Zargar et al	Kashmir	2000	7.0	23.7
Gopalan	Nutrition Foundation of India	1998 Upper Strata	32.2	50
		Middle Class	16.2	30.3
		Low S.E.group	7.0	27.8
		Poor urban slum	1.0	4.0
District nutrition	Food and Nutrition Board	1998 Rural Urban	0..3	0.7
Profiles survey			0.4	0.7
National Family				
Health survey		1998-99	-	2.3
Mohan et al	Chennai urban	2001 Obesity	22.8	31.8
	population study	Abdominal Obesity	21.5	36.5
Deshmukh et al	Rural Wardha	2006 Obesity	5.1	5.2
Abdominal Obesity			7.6	8.7
Kiran Kumar et al	Nellore (Urban)	2011 Obesity	49.5	50.5
Abdominal Obesity			63.2	73.4

Using the formula $n = (Z\alpha)^2 pq / L^2$ sample size was calculated to be 533.33 which has been rounded to 550 subjects in the age group of 20-60 years from the study area. The ethical clearance for this study was obtained from Institutional Ethical Committee of Narayana Medical College. A pilot study was done among 30 individuals of the urban population in areas other than UHC field practice area. The study was conducted from October 2010 to August 2011 by using Systematic Random Sampling method. The total numbers of houses present in the UHC field practice area of (5-6 sq. km approx) were 5902 with a population of approximately 25127, of 12588 males and 12539 females.

For a wide coverage of the community, it was decided to study every 8th house. To ensure random selection, the following procedure of UIP surveys using a currency note was applied. The center of the area was identified and the number of streets joining at the centre were numbered. One of the streets joining at the center was selected randomly, using the last digit of the serial number of the currency note. All the houses in the street were numbered. Again after reaching the midpoint of this street, a starting household was selected, using the last digit of the currency note. The direction to proceed right or left is chosen randomly. Here the next household (8th) was selected going to the right hand side. In case if the 8th house is locked then the next adjoining house i.e 9th house was chosen. The same method was followed in all areas, till the sample size was attained. If more than one adult was found in a family, then the elder one was included in the study the reason being to ensure more coverage of sampled houses and thereby wider geographical coverage. Data was collected by interview method using pre-designed and pre-tested questionnaire and administered to all of the 550 persons. All the study subjects were explained in detail about the purpose and methodology of the study and were fully assured of strict confidentiality. Informed oral consent was obtained.

Inclusion criteria :

Study subjects aged between 20-60 years residing for period of 6 months or more in field practice area of UHC, Saraswathi Nagar.

Exclusion criteria :

1. Study subjects who did not give consent for participation in this study.
2. Visitors and houses which were locked during the period of this study.
3. All pregnant women, lactating mothers, visitors, bed-ridden patients, persons with anatomical abnormalities, hypothyroid patients, ascitis patients, liver failure patients.

Subjects satisfying the eligibility criteria of the study were interviewed face to face by using the questionnaire. Questionnaire had details on socio-demographic characteristics of the individual age, religion, caste, occupation, nature of work, educational status, type of family, per capita income and social class and family history of

obesity. Modified B.G.Prasad's socio - economic status scale was used to classify study subject's socio-economic status. It is updated for per capita monthly income according to the All India Wholesale Price Index (AIWPI) for the month of August 2010. The various classes of this scale are I – Upper High (> Rs.7400), II - High (Rs.3700-7399), III - Upper Middle (Rs.2200-3699), IV - Lower Middle (Rs. 1100-2199), V- Poor (Rs.370-1099), VI-BPL or Very poor (<Rs.370). Nature of work was assessed and accordingly the study population were classified as sedentary workers, moderate workers and heavy workers. Following workers were considered under these headings..Sedentary workers: Male – Teacher, Tailor, Barber, Executive, Shoe-maker, Priest, Retired person, Land lord, Peon, Post man and others. Female – Teacher, Tailor, Executive, House wife, Nurses and others. Moderate workers : Male – Fisherman, Basket maker, Potter, Goldsmith, Agriculture labour, Carpenter, Mason, Rickshaw puller, Electrician, Fitter, Turner, Welder, Industrial labour, Coolie, Weaver, driver and others. Female – Maid servant, Coolie, Basket maker, Weaver, Agriculture labour, Beedi maker, etc. Heavy workers: Male – Wood cutter, Black smith, Mine worker, Gang man, etc. female – Stone cutter. Then the section on personal history had dietary history regarding consumption of energy rich foods, fruits, vegetables, salt intake, type of oil consumed, H/o regular physical exercises, H/o emotional disturbances, H/o alcohol intake, H/o smoking and any drugs used by the subject which cause obesity, etc. Consumption of energy dense foods like sweets, refined foods, papads, paneer, pizza, cheese, burgers, halwa, creams, fats, etc. was considered if the subject has them every day or alternate days. Salt intake was calculated by finding out the total quantity the family uses in a month, which was divided by the number of family members to find out daily consumption per head. Regular physical exercise includes mild or moderate or hard or very hard physical activity. Mild or moderate physical activity for at least 30 minutes which includes, fast walking on flat surface, cycling on level surface, gardening (raking leaves, weeding, planting trees etc), house painting, mopping the floor, cleaning windows, golf – walking and pulling/carrying clubs, fishing, ball room dancing, light/moderate strength-developing exercise, volley ball, snorkeling, badminton etc. Hard/very hard physical activity (at least for 10 minutes) includes jogging, cycling up hill, chopping wood, scrubbing floors, moving heavy furniture, heavy digging in the garden, swimming, soccer, basket ball etc. The emotional disturbances of the study subject was ascertained by asking if the study subject had emotional disorders like depression, anxiety etc for which he/she was referred to the Consultant psychiatrist/PG-psychiatry for further confirmation and diagnosis for consideration for the study.

Consumption of smoking form of tobacco by a study subject in the past 6 months was considered as a smoker. The types of tobacco consumption considered included smoked ones like cigarettes, beedis, chuttas and cigars. Consumption of any form of alcohol at least once a week in a month in the past 6 months was considered as alcohol consumption.

The history of any non-communicable diseases history like hypertension, diabetes, coronary heart disease, stroke, varicose veins and hernia were also recorded. The family history of (h/o) obesity was elicited by enquiring about cases of obesity in the study subject's family diagnosed by a doctor and also assessing the same if present. Obesity was assessed by measuring weight, height, waist circumference and hip circumference. From these, body mass index (BMI) and waist hip ratio (WHR) were calculated. Measurement of blood pressure for all subjects with the recommended measurement protocols laid down by WHO, were done.

STATISTICAL METHODS

The collected data was analyzed with SPSS (version17.0 for windows). The prevalence rate of obesity and Chi-square test was done to find out association of obesity with various other factors in this study. Multivariate logistic regression analysis was done to assess the independent association of factors with obesity.

RESULTS

Out of total 550 study population, 272 (49.5%) were males and 278 (50.5%) were females. Maximum numbers 174 (31.6%) of study subjects were in the age group 31-40 years.(Table-2) Majority 465 (84.6%) of the study subjects were Hindus, followed by Christians 60 (10.9%) and Muslims 25 (4.5%). Major proportion 245 (44.5%) of the sample belonged to Forward/Other communities (OC), followed by Backward communities (BC), 154 (28.0%), Scheduled communities (SC), 112 (20.4%) and Scheduled Tribes 39 (7.1%). Among females, 83% were Housewives and 10.8% were Unskilled workers. Among males, 33.8% were self-employed/ clerical/ shop owners, 18.8% were unskilled workers, 15.4% were Skilled workers, 13.6% were Semi skilled workers, 11% were Unemployed and 7.4% were Semi professionals. Among the study population 426 (77.5%) were Moderate workers, 64 (11.6%) were Sedentary workers and 60 (10.9%) were Heavy workers. Out of total 550 study population 146 (26.5%) of them were illiterates and the rest

Table 2: Age and gender distribution of study population (n = 550)

Age groups	Gender				Total	
in years	Male		Female			
	No.	%	No.	%	No.	%
20-30	62	22.8	83	29.9	145	26.4
31-40	85	31.3	89	32.0	174	31.6
41-50	69	25.4	66	23.7	135	24.5
51-60	56	20.6	40	14.4	96	17.5
Total	272	49.5	278	50.5	550	100

404 (73.5%) are literates. Among literates 97 (24%) did High school certificate, 95 (23.5%) were Graduate or Post Graduate, 88 (21.8%) did Middle school certificate, 70 (17.3%) did Primary school and 54 (13.4%) did Intermediate Post High School Diploma and none were holding Professionals or Honours. As per modified B.G. Prasad's socio economic status scale only 0.2% were below poverty line, 13.8 % were poor, 34.4% belong to lower middle class, 27.5% belong to upper middle class, 18.7% belong to high class and 5.5% belong to upper high class.

Table 3: Categories of obesity among study subjects (n = 550)

Criteria	BMI	No.	(%)
Under weight	< 18.50	43	(7.8)
Normal	18.50-22.99	119	(21.6)
At risk	23.00-24.99	96	(17.5)
Obesity stage-1	25-29.99	221	(40.2)
Obesity stage-2	> 30	71	(12.9)
Total		550	(100)

According to BMI[4,6] 292 (53.1%) study subjects were found to be obese thus giving an obesity prevalence of 53.1%. 119 (21.6%) of the study subjects were having normal BMI, 221(40.2%) had stage I obesity, 71(12.9%) had stage II obesity, 96(17.5%) were at risk of obesity, and only 43 (7.8%) were underweight.(Table-3)

As per waist circumference,[4,6] 376 (68.4%) of the study subjects were obese and 174 (31.6%) were non obese, giving an abdominal obesity prevalence of 68.4% . Females (73.4%) were more obese than males (63.2%) and the difference was found to be statistically significant. (p = 0.011) (Table-4)

Table 4: Distribution of study subjects as per Abdominal Obesity (waist circumference) (n = 550)

	Obese		Non-obese		Total	
Sex	No.	(%)	No.	(%)	No.	(%)
Male	172	(63.2)	100	(36.8)	272	(49.5)
Female	204	(73.4)	74	(26.6)	278	(50.5)
Total (100%)	376	(68.4)	174	(31.6)	550	

X^2 = 6.544; df = 1 ; p = 0.011

According to waist hip ratio (WHR) [4-6], 289 (52.5%) of the study subjects were obese and 261 (47.5%) were non obese, giving an abdominal obesity prevalence of 52.5%. Females (67.6%) were found to be more obese than males (37.1%) and the difference was found to be statistically very highly significant. (p < 0.001) (Table-5)

The proportion of obesity was seen increasing with age upto 5th decade. Highest prevalence rate (61.5%) was observed in 41–50 years age group and the lowest prevalence rate (42.8%) was seen in age group of 20–30 years. The difference between different age groups was statistically

Table 5: Distribution of study subjects as per Abdominal Obesity (waist hip ratio) (n = 550)

	Obese		Non-obese		Total	
Sex	No.	(%)	No.	(%)	No.	(%)
Male	101	(37.1)	171	(62.9)	272	(49.5)
Female	188	(67.6)	90	(32.4)	278	(50.5)
Total (100%)	289	(52.5)	261	(47.5)	550	

X^2 = 51.269; df = 1 ; p < 0.001

significant. (p = 0.017) The prevalence of obesity was seen to be highest in OC (61.6%) community and lowest in ST (25.6%) community. The prevalence of obesity was observed to be decreasing from higher community to lower community and the difference was found to be statistically very highly significant. (p < 0.001) Highest obesity (67.4%) was seen in skilled workers, and lowest (33.3%) in the unemployed. The difference of prevalence of obesity in different work groups was statistically highly significant. (p = 0.001) The prevalence of obesity was 31.7% in heavy workers, 54.50% in moderate workers, and 64.10% in sedentary workers. The prevalence of obesity was highest in sedentary workers and lowest in heavy workers. The difference of prevalence of obesity in different workers was statistically highly significant. (p = 0.001) The prevalence of obesity was 57% in nuclear families, 44.9% in joint families and 43.8% in three generation families. Highest obesity was seen in nuclear family study subjects and lowest in three generation family study subjects. The prevalence of obesity in people with different types of families was statistically significant. (p = 0.042) The prevalence of obesity was highest (73.3%) seen in the upper high class, obesity gradually decreases and was nil in very poor class. The differences in the prevalence of obesity among these various socio economic classes was statistically highly significant. (p = 0.002) Out of 267 study subjects who gave positive family history of obesity, 64.4% were obese whereas out of 283 study subjects who gave negative family history of obesity 42.4% were obese. The difference was statistically very highly significant.(p < 0.001) 67(64.4%) subjects had obesity out of 104 study subjects who were consuming energy rich foods whereas 225(50.4%) had obesity out of 446 study subjects who were not consuming energy rich foods. The difference was found to have a significant statistical association. (p = 0.005) 218 study subjects were having emotional disturbances, of whom 127(58.3%) had obesity. Of the 332 subjects who did not have any emotional disturbances, 165(49.7%) were obese. The difference was statistically significant. (p = 0.049) About 92 subjects who consumed alcohol, and among 33.7% were obese. Out of 458 who did not consume alcohol, 57% were found to be obese. The difference was found to be statistically very highly significant. (p < 0.001) There were 95 subjects who were smoking and among them 41.1% were obese. Out of 455 subjects who did not smoke, 55.6% were found to be

obese. The prevalence of Obesity was high among subjects with no history of smoking and this difference was found to have a significant statistical association.(p = 0.010) Out of 292 obese subjects, 18.5% were having Hypertension whereas out of 258 non-obese subjects,10.5% were having Hypertension. The prevalence of Hypertension is high among subjects with Obesity. The difference was found statistically highly significant. (p = 0.008) Out of 292 obese subjects, 15.1% were having Diabetes whereas among 258 non-obese subjects, 8.1% were having Diabetes. The prevalence of Diabetes is high among subjects with Obesity. The difference was found to be statistically significant. (p = 0.012) The overall Diabetic prevalence in the present study was 11.8%. The association of obesity with other risk factors viz. sex , religion, education, dietary habits, salt intake and regular physical exercise was not found to have a significant statistical association. (p > 0.05) (Table-6). The independent factors associated with the prevalence of obesity were

Table 6 : Risk Factors associated with obesity (n = 550)

Variables	Obese (%)	Non-obese (%)	X^2	p value
Age (years)				
20-30	62(42.8)	83(57.2)		
31-40	95(54.6)	79(45.4)		
41-50	83(61.5)	52(38.5)	10.235	0.017
51-60	52(54.2)	44(45.8)		
Caste				
OC	151(61.6)	94(38.4)		
BC	79(51.3)	75(48.7)		
SC	52(46.4)	60(53.6)	21.172	< 0.001
ST	10(25.6)	29(74.4)		
Occupation				
Semi-professionals	12(60.0)	8(40.0)		
Self-employed/Clerical	58(58.0)	42(42.0)		
Skilled worker	29(67.4)	14(32.6)		
Semi-skilled worker	17(37.8)	28(62.2)	23.645	0.001
Unskilled worker	31(38.3)	50(61.7)		
Unemployed	10(33.3)	20(66.7)		
Housewife	135(53.1)	96(41.6)		
Nature of work				
Heavy	19(31.7)	41(68.3)		
Moderate	232(54.5)	194(45.5)	14.472	0.001
Sedentary	41(64.1)	23(35.9)		
Type of Family				
Nuclear	217(57.0)	164(43.0)		
Joint	40(44.9)	49(55.1)	7.460	0.042
Three generation	35(43.8)	45(56.3)		
Socio-economic class				
Upper high	22(73.3)	8(26.7)		
High	64(62.1)	39(37.9)		
Upper middle	84(55.6)	67(44.4)		
Lower middle	94(49.7)	95(50.3)	18.753	0.002
Poor	28(36.8)	48(63.2)		
Very poor/BPL	0(0)	1(100.0)		
Family H/o obesity				
Present	172(64.4)	95(35.6)	26.740	< 0.001
Absent	120(42.4)	163(57.6)		

Variables	Obese (%)	Non-obese (%)	X^2	p value
Intake of Energy Rich Foods				
Yes	67(64.4)	37(35.6)	6.613	0.005
No	225(50.4)	221(49.6)		
Emotional Disturbances				
Present	127(58.3)	91(41.7)	3.870	0.049
Absent	165(49.7)	167(50.3)		
H/o Alcohol Intake				
Present	31(33.7)	61(66.3)	16.688	< 0.001
Absent	261(57.0)	197(43.0)		
H/o Smoking				
Present	39(41.1)	56(58.9)	6.682	0.010
Absent	253(55.6)	202(44.4)		
Hypertension				
Present	54(66.7)	27(33.3)	7.030	0.008
Absent	238(50.7)	231(49.3)		
Diabetes				
Present	44(67.7)	21(32.3)	6.310	0.012
Absent	248(51.1)	237(48.9)		

identified by performing multivariate logistic regression analysis. Multivariate logistic regression model showed age (> 30 years),community group(OBC, ST, SC),Family(Three generation), Socio-economic class(Middle class), positive Family h/o obesity, positive Intake of energy rich foods, Emotional disturbances (present), positive H/o alcohol intake, H/o smoking (present),Hypertension(present) and Diabetes(present) were independently associated with prevalence of obesity. (Table-7).

DISCUSSION

According to Body Mass Index (BMI) [4,6] in the present study, about 292 (53.1%) study subjects out of 550(100%) were found to be obese thus giving an overall prevalence of obesity of 53.1%, 49.6% in males and 56.5% in females The prevalence of at risk of obesity was 17.5%. Similar observations were reported by Gupta R, et al.[7] Bansal M, et al,[8] ICMR Task force [9] and WHO Sentinel Surveillance Systems for CVD in Indian Industrial Populations [10] had findings consistent with the present study. Kokhar, et al found obesity prevalence of 70.30% and 75.09% among pre and postmenopausal women in Punjab which was higher than the current study. [11] Studies by District nutrition profiles survey [12] and Deshmukh PR , et al[6] in rural part of Sewagram, Wardha district reported very low prevalences of obesity in men and women. In the review of about fifty studies done in India and abroad, the prevalence of obesity is minimum in Dibrugarh in Assam (India) i.e., 0.5% [10] (2001-03) and maximum in Micronesian island of Naru i.e., 85% for males and 93% for females in 2004.[13]

As per waist circumference,[4,6] 376 (68.4%) of the study subjects were obese and 174 (31.6%) were non obese, giving an abdominal obesity prevalence of 68.4% . Females were more obese (73.4%) than males (63.2%). Similar observations were noted by Kokhar, et al where prevalence of obesity was 75.15% and 89.05%, in pre and postmenopausal women, respectively,[11] This was contradicted by other studies one by WHO Sentinel Surveillance Systems for CVD in Indian Industrial Populations [10] which reported prevalence of 0.7% in Dibrugarh, Assam and 52% in Hyderabad, and the other by Gupta,et al[9] who noted a prevalence of abdominal obesity of 33.2% in 2001 and 45% in 2003 in Rajasthan. This could be due to higher value cut off values used for waist circumference.

According to waist hip ratio (WHR) [4-6], 289 (52.5%) of the study subjects were obese and 261 (47.5%) were non obese, giving an abdominal obesity prevalence of 52.5% . Females (67.6%) were found to be more obese than males (37.1%). Similar observations were made by Kripa R , et al [15] High prevalence of abdominal obesity(60%-88%) were noted in the studies by Khokhar,et al, [11] Gupta R, et al [7] ICMR Task Force Project in urban Delhi, rural Haryana, urban Rajasthan, [9] Reddy NK in Andhra Pradesh [9] and Prabhakaran D ,et al [14] and low prevalence 21% of abdominal obesity in rural Rajasthan [9] which differ with the findings of the present study. The prevalence in the above studies is probably high due to considering low cut off values for defining WHR.

The present study showed that obesity increased with advancing age, till 5th decade. Highest prevalence rate of 61.5% was observed in the age group of 41–50 years. Difference between the different age groups was found to be statistically significant. ($p < 0.05$) Similar observations were noted in the studies by Asthana S, et al, [16] Gopinath N,

Table 7: Multivariate logistic regression analysis

Variables	B	Std.error	d.f.	Sig	Exp(B)	95% Confidence Interval Lower Bound	Upper Bound
Age (years)							
<30	1						
>30	-0.667	0.234	1	0.004	0.513	0.324	0.812
Sex							
Male	1						
Female	-0.340	0.321	1	0.290	0.712	0.380	1.336
Religion							
Hindu	1						
Muslim	-0.042	0.318	1	0.895	0.959	0.514	1.790
Christian	0.159	0.558	1	0.776	1.172	o.392	3.502
Caste							
General	1						
OBC/ST/SC	0.473	0.242	1	0.041	1.605	0.998	2.580
Occupation							
Employed	1						
Unemployed/Housewife	0.447	0.296	1	0.132	1.563	0.875	2.793
Nature of work							
Heavy	1						
Moderate	-0.875	0.478	1	0.067	0.417	0.163	1.065
Sedentary	-0.296	0.342	1	0.386	0.743	0.380	1.453
Education							
Illiterate	1						
Literate	-0.327	0.234	1	0.163	0.721	0.455	1.141
Type of family							
Joint	1						
Three generation	-0.586	0.269	1	0.029	0.556	0.329	0.942
Nuclear	-0.402	0.277	1	0.147	0.669	0.388	1.152
Socio-economic class							
Poor	1						
Middle class	-0.890	0.345	1	0.010	0.410	0.209	0.808
Rich	-0.277	0.244	1	0.256	0.758	0.470	1.223
Family H/o obesity							
No	1						
Yes	-1.105	0.196	1	0.000	0.331	0.225	0.486
Intake of Energy rich foods							
Yes	0.651	0.231	1	0.005	1.918	1.221	3.014
No	0						
Emotional disturbances							
No	1						
Yes	-0.410	0.202	1	0.042	0.664	0.447	0.986
H/o Alcohol intake							
No	1						
Yes	0.738	0.332	1	0.026	2.092	1.092	4.006
H/o Smoking							
Yes	1						
No	-0.061	0.320	1	0.849	0.941	0.502	1.762
Hypertension							
Yes	0.655	0.253	1	0.010	1.924	1.171	3.161
No	0						
Diabetes							
Yes	0.686	0.280	1	0.014	1.986	1.146	3.440
No							

et al, [17] NFHS – 2 , India, [18] NFHS – 3, India, [3] Shukla HC, et al, [20] Naidu AP, et al. [21]

In the present study, the prevalence of obesity was 49.6% among males and 56.5% among females. There was no significant statistical association between obesity and sex ($p > 0.05$) . Similar observations was made by NFHS-3 ,[3] Ram K, et al [15] indicating that obesity was more prevalent among females than males. Previous research on obesity in India has found the prevalence of obesity to be higher among women. [23] It was noted, that in Asia the prevalence of obesity in 18-69 years age group was 12.4% in males and 16.5% in females. [24]

This study showed that out of 465 Hindus 247 (53.1%) were obese, in Muslims 14(56%) out of 25 were obese, and among Christians, 31(51.7%) out of 60 were obese. However, this difference was not significant ($p > 0.05$). Poluru R, et al (1998-99) conducted a review study reported that, Muslim women in Maharashtra and non-Hindu (Christian) women in Goa were more likely (odds = 1.4) to be overweight or obese than the Hindu-OC women, which was similar to the findings of the present study. [27]

The prevalence of obesity was highest in OC and lowest in ST community and it was seen decreasing from higher to lower communities. Statistically the difference was very highly significant, ($p < 0.001$). Poluru R, et al, reported that, Hindu-SC/ST women in Gujarat were less likely to be overweight/obese than the Hindu-OC women. [27]

In the present study it was observed that the prevalence of obesity was highest in skilled workers, and lowest in the unemployed and the difference was statistically highly significant.($p < 0.01$) Similar observations were reported by Wang C, et al [28] and Amegah AK et al.[29]

In the present study the prevalence of obesity was highest in sedentary workers(64.10%) and lowest(31.7%) in heavy workers and the difference was statistically highly significant. ($p < 0.01$) Similar observations were made by Gopinath N, et al ,[17] Naidu AP, et al [21], Wang C, et al [28] and Amegah AK, et al[29] in their studies.

In the present study the highest prevalence (57.7%) of obesity was observed in the high school certificate group and lowest prevalence (45.9%) in the illiterates. The difference in the prevalence rates of obesity among different educational status was not statistically significant. Similar observations were made by Poluru R ,et al, [27] Agrawal P, et al, [23] NFHS-2 data,[18] Jitnarin N ,et al [30] and Ziraba AK et al [31] in their studies.

In this study, the prevalence of obesity was high in upper socioeconomic groups compared to lower socio economic groups .The differences in the prevalence of obesity among these socio economic groups was statistically highly significant ($p < 0.01$).Mohan V,et al [31] in a study in Chennai, Shetty PS [26] and other studies [16,19] reported similar findings. Data from India show that higher-income groups consumed a diet with 32% of the energy from fat while the lower-income groups consumed only 17% energy from fat. More recent dietary surveys in Delhi also confirm that the upper income groups in urban India currently consume higher levels of energy from fat as compared with the urban poor or rural populations. [32]

In the present study it was observed that out of 446 study subjects who were taking mixed diet 51.3% had obesity whereas out of 104 vegetarians, 60.6% had obesity. The difference however was statistically not significant, ($p > 0.05$). Shi Z, et al in a study in Jiangsu Province China reported that, the vegetable-rich food pattern (whole grains, fruits and vegetables) was independently associated with higher risk of obesity/central obesity in Chinese adults in both genders which was similar to the findings of the present study.[26]

In the present study there was a significant statistical association ($p < 0.05$) between obesity and consumption of energy rich foods. According to WHO studies conducted in 1991 showed that, in the past 20 years the prevalence of obesity has tripled in developing countries that have been adopting a western life style involving decreased physical activity and over consumption of cheap, energy dense foods.[2] Similar findings were reported by Naidu AP, et al [2]1 and Shetty PS [26] in their studies.

In the present study it was observed that obesity was more in subjects without physical exercise .The difference was statistically not significant. ($p > 0.05$) Similar observations were made by Wang C, et al [28] and Bilal A, et al [32] in their studies.

The prevalence of Obesity was high among subjects with no H/o alcohol intake and the difference was statistically very highly significant. ($p < 0.001$) Fan JG, et al from a study in Shanghai urban population, China ($n = 3953$) reported similar findings that abdominal obesity was 36.2% in non-drinkers and 28.8% among drinkers ($p < 0.01$).[33]

The prevalence of obesity was high among subjects who do not smoke and the difference was statistically significant. ($p < 0.05$) Studies by Sabanayagam C, et al [34] among Chinese population in Singapore, Jitnarin N, et al [30] in urban Thailand, Shukla HC, et al[20] in Mumbai city, Erem C, et al [19] in Turkey and Amegah AK,et al[29] in Cape Coast, Ghana reported similar observations which were consistent with the findings of the present study. This may be due to decreased appetite following smoking.

The prevalence of Hypertension was high among the subjects with obesity and the difference was statistically highly significant. ($p < 0.01$) Similar observations were made by, Deshmukh PR,et al[6], Gopinath N, et al[9], Erem C, et al[19], Nawaz H, et al[22], Misra A, et al[35] and Mohan V, et al[31] in their studies.

The prevalence of Diabetes was high among subjects with Obesity and most of the subjects were unaware of their Diabetic status. The difference was statistically

significant.($p < 0.05$) Similar observations were made by Gopinath N,et al[17] and Nawaz H,et al.[22] There is a positive association between obesity and risk of developing non-insulin dependent diabetes mellitus. The risk of non-insulin dependent diabetes mellitus increases continuously with increase in body mass index and decreases with weight loss. Abdominal obesity is important in the development of insulin resistance.

CONCLUSION

The present study showed a high prevalence of obesity among adults in urban area in Nellore city. Two priority interventions important in preventing the development of obesity are increasing levels of physical activity and improving the quality of diet. This can be applied, either to the whole population or to the high risk population.

Promotion of relatively low intensity, long duration physical activity can be conveniently incorporated into daily life. Activity should be enjoyable in order to encourage regular participation and to discourage sedentary behaviour.

Promotion of healthy diet that is low in fat, high in complex carbohydrates and containing large amounts of fresh fruit and vegetables . The prevention and management of obesity are not solely the responsibility of individuals, their families, health professionals or health service organizations and a commitment by all sectors of society is required.

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SUPERVISED EXERCISE TRAINING IMPROVES FUNCTIONAL CAPACITY DURING PHASE II CARDIAC REHABILITATION IN POST CABG PATIENTS

N. Venkatesh[a], T. Senthil Kumar[a], S. Sridevi[a]

ABSTRACT:

Background: *Cardiac rehabilitation includes the essential component of graded exercise training with growing demand on individualized training methods to enhance the benefits of such training programs. Amidst the limitations in supervised training adherence, recent trends of evidence based practice and younger age of surgical candidacy entrusts to explore the benefits of simple and systematic exercise training in Indian settings.*

Objective: *To evaluate the benefits of simple and individualized training on functional improvement among post CABG patients.*

Methodology: *All the patients undergoing CABG at the super specialty center were screened for inclusion in this study. The eligible candidates were randomized into experimental (EXP) and Control (CON) group. The Phase I training remained same for the patients of both groups. Before discharge, the patients in control group were given routine care with counseling to continue self-monitored exercise. The subjects in Experimental group attended individualized training sessions under supervision. The functional capacity was assessed for patients in both groups at the time of discharge and after 12 weeks follow up.*

Results: *The pre-post walking distance of EXP and CON group were 140 -433 and 143-268 respectively .The patients of EXP group were found to have significant improvement in their functional capacity following supervised exercise training than Control group($P < 0.05$)*

Conclusions: *The adherence to exercise program was a major limiting factor in this study. The patient who attended the supervised exercise program (EXP), had a significant improvement in their functional capacity in comparison to self-monitored training (CON) group.*

Key Words: *Cardiac Rehabilitation, coronary artery bypass grafting, phase II, supervised training,*

Mesh Words*: Exercise capacity, exercise training, six minute walk distance, physiotherapy*

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INTRODUCTION:

Over recent decades, coronary artery bypass grafting (CABG) has become one of the common treatment method for patients with Coronary Artery Disease (CAD) and after CABG, cardiac rehabilitation programs are considered important essential component.[1] A Cardiac Rehab program consisting of exercise training, life style changes and psycho-social interventions are emphasized by several prominent health agencies.[2] The benefits of mortality, morbidity, functional independence and Quality of Life have been explored and found have a reasonable health benefits with such systematic training[1, 3, 4] .

The CR programs were also found to have cost effectiveness.[5-7] Even low intensity exercises were safe to be done unsupervised for low risk patients,[8] the extent of benefits of a tailor-made, supervised progressive training is sparsely available in Indian settings. The compliance of patient to supervised training has remained a challenge due to multiple factors. Tailoring the program to the patient's needs empowers the patient and improve adherence.[9] India has a large population of coronary artery disease (CAD),[10] but this concept has not gained momentum in India. Hence, this study on the effectiveness of Supervised Exercise Training during Phase II Cardiac Rehabilitation following CABG was taken up.

MATERIALS AND METHODS:

This was an exercise based interventional study. The study was approved by the Institutional Ethical Committee (IEC) of Sri Ramachandra University, Chennai. Since the expected prognosis is anticipated in 50% of subjects who participate in the study and the deviation may be 20% and with spontaneous improvement of control group being 25%, at the predicted significance at 5%, the total participants

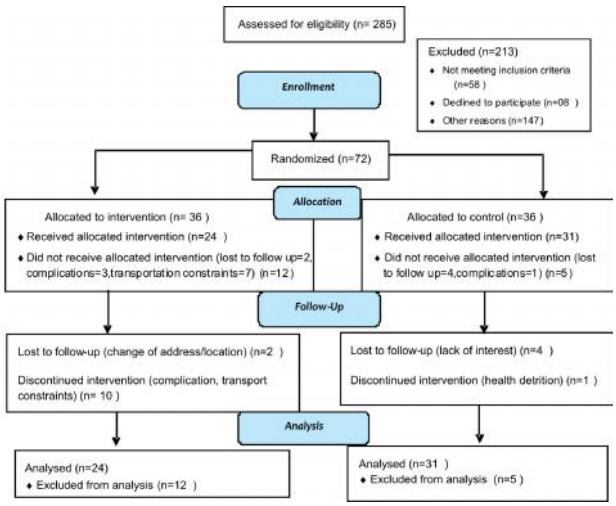

Fig. 1: Scheme of Study

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was calculated to be 58 (Power analysis). Considering the attrition and loss to follow up, a sample size was determined to be of 70 subjects (35 subjects in intervention and 35 subjects in control group).The patients of low and moderate risk group were included for this study. The risk stratification was done in accordance to AACVPR Guidelines for Cardiac Risk Stratification. About 285 patients were screened for study inclusion and 72 patients who met the criteria and consented were enrolled in this study (Figure 1).

PROCEDURE: The patients meeting the inclusion criteria were included for this study after obtaining, due informed consent. All patients received routine post-operative management in both the groups which included bronchial hygiene, breathing exercise and graded ambulation. The usage of six minute walk test has been tested by many and found to be suitable for assessment of functional capacity in many conditions including, cardiac patient.[11,12] The exercise capacity of patients was assessed with a six minute walk test at the time of discharge, as per standard protocol established.[13-17] Patient's exertion level (RPE) was monitored with Borg's scale and the distance was noted. The pulse rate (HR) and oxygen saturation (SpO2) was monitored before, during and after the completion of test. The heart rate, Rate of Perceived Exertion (RPE) and Spo2 recorded was used as basis for exercise prescription in phase II rehabilitation. They were randomized into the intervention or control group as per computer generated random numbers. Patients of both the groups received exercise counseling before discharge.

Exercise Intensity: The exercise prescription for the patients was moderate intensity with 55%–75% of Target heart rate.[18] The exercises were started with moderate intensity level i.e.55% of Target heart rate ; over and above the resting heart rate will be considered as the intensity of training.[19] The patients were trained with Borg's RPE method for intensity monitoring in Phase I, so as to be used by the patients during phase II rehabilitation. The RPE level of mild to moderate exertion[9-11] was used in this study

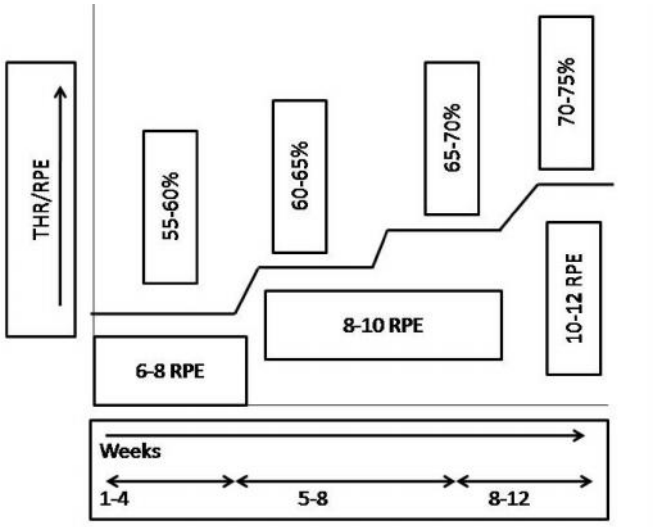

Fig.2: Exercise intensity progression protocol

Exercise Protocol: The patients in Interventional group underwent a set of structured exercise program at out- patient physiotherapy department 2-3 sessions /week for a period of 12 weeks. They performed low to moderate intensity exercise training, for at least 20 -30 minutes which consisted of walking, upper and lower extremity movements with 1 to 2.5 kg of resistance. All sessions had adequate warm up and warm down for about 15-20 minutes. The total duration of exercise lasted up to 30-50 minutes. The exercise was supervised and vitals were monitored through the sessions. The Pulse rate, Blood Pressure, SpO2, Respiratory rate and Rate of Perceived Exertion (RPE) were monitored during training. The intensity progression was based on Target Heart Rate (THR) and RPE over a period of 12 weeks (Fig. 2).

The patients in control group continued to follow exercise trained at time of discharge advice at home. The patients, at home was asked to record in an activity log. After 12 weeks, patient of both the groups were tested for their changes in exercise capacity with six minute walk test and all parameters were noted down.

As most patients were unable to give consent or unable to maintain regular follow up for outpatient therapy even though they all showed interest in exercise counseling, the barriers to Phase II program participation was also noted down for further analysis.

ANALYSIS AND RESULTS

Among 72 patients enrolled for the study, 24 in intervention group and 31 in control group completed the study. The functional capacity was measured by standard six minute walk test (SMWT) at time of discharge and after 12 weeks of training period was taken up for analysis.

The statistical analysis showed that the patients were similar in terms of age, number of dyslipidemics, smokers,diabetics and duration of Intensive care Unit (ICU) stay between the two groups (Table 1). The difference in

Table-1: Baseline Characteristics of the study sample

Characteristics	Experimental group	Control group	t-Value	Level of Significance
Age (Mean)*	58.28	57.77	-0734	0.466 (P > 0.05)
Dyslipidemia	22	23	-1.679	0.099 (P > 0.05)
smoking	9	11	-0.151	0.880 (P > 0.05)
Diabetics	20	26	0.052	0.958 (P > 0.05)
ICU stay (Days)*	3.52	3.48	0.122	0.904 (P > 0.05)
Obesity (BMI)	20	19	0.4.22	0.782 (P > 0.05)

*The values are count data unless otherwise stated

functional capacity measured as SMW distance (in meters) was statistically insignificant at pre and post-training levels, when compared between various risk factors (Table 2, Fig.3 & 4).There was an improvement in both the control and experimental groups at the end of the study period ; There was a statistically highly significant improvement in the experimental group than the control group of patients in terms mean difference in functional capacity (SMWD) compared from the baseline of study (Table 3 & Fig.5).

Table-2: Pre-Post Training comparison of functional capacity by risk factors

	Pre-training	Post-training	t-value	Level of Significance
Diabetic	144	332	0.033*	0.857* (P>0.05)
Non Diabetic	127	380	1.545#	0.219# (P>0.05)
Smoker	139	344	0.05*	0.824* (P>0.05)
Non smoker	143	337	0.53#	0.473# (P>0.05)
Obese	139	309	0.692*	0.0409* (P>0.05)
Non obese	149	357	0.372#	0.059# (P>0.05)

*Pre Comparison, # Post Comparison

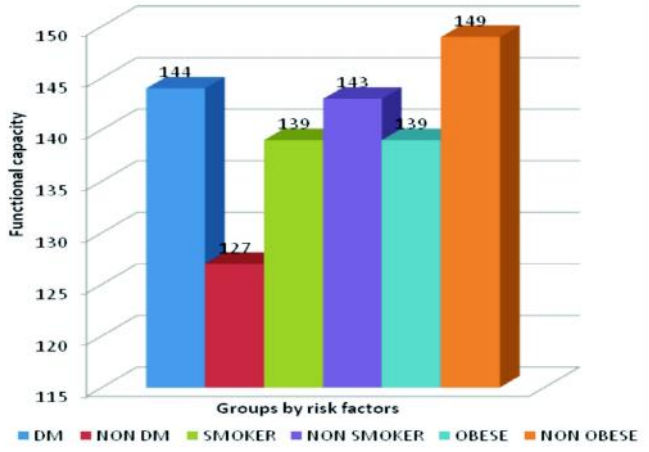

Fig. 3: Pre-Exercise training Comparison of Functional Capacity by risk factors

Table-3: Pre-Post-Training comparison of functional capacity between Control and Experimental groups

Pre-training	Group	Mean ± SD	t-value	P-Value
	Control	143 ± 33.29	0.332	0.741 (P>0.05)
	Experimental	140 ± 28.16		
Post-training	Control	268 ± 53.45	13.458	0.000 (P<0.001)
	Experimental	433 ± 30.90		

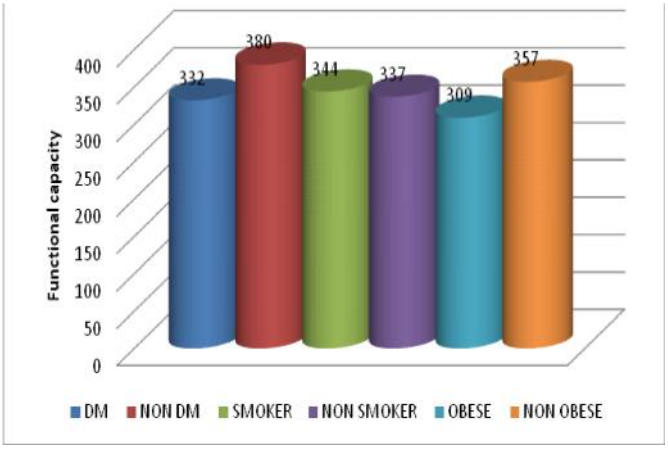

Fig.4: Post-Exercise training Comparison of Functional Capacity by risk factors

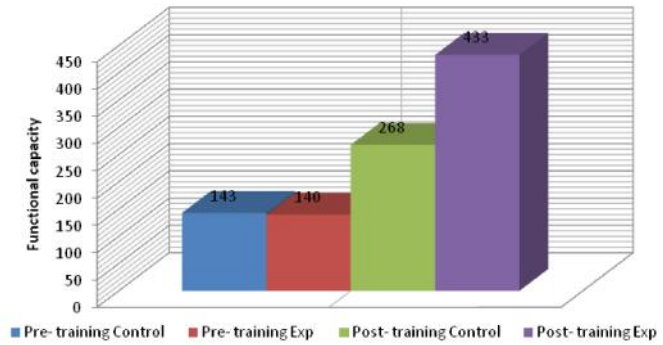

Fig.5: Pre-Post Exercise training Comparison of functional capacity between groups

DISCUSSION

It is generally accepted that systematic training would result in better results. In the present study the samples were similar in their baseline exercise capacity and their cardiac risk levels. The supervised exercise training group showed statistically significant improvement in their functional capacity than the control group. None of the patients showed any adverse response during exercise training throughout the study period; this study adds to the current body of evidence to develop a feasible simple, tailor-made exercise program in phase II, which could effectively improve the exercise capacity following CABG.[8]

The experimental group patients have shown better improvement than the control group subjects which in accordance to previous studies.[20-22] About 12 and 5 patients didn't complete the exercise training in the experimental and the control group respectively. As like in other studies, loss to follow up was a hurdle in effective and complete implementation of the program.[23] The commutation constraints and Lack of patient interest were the common reasons for loss to follow up in majority of patients, which has been noted in previous studies too.[24] Loss to follow up was more in experimental groups shows the need for more emphasis on patient and family education[25,26] and need for

implementation of insurance coverage for cardiac rehab programs as in western parts of the world.[6, 7, 27] In this study sample had similar characteristics except for number of diabetics was not similar, but the difference was statistically insignificant. Moreover the functional capacity was also not statistically significant in this study, during pre training. However the current result needs to be verified with equal number of diabetics in both the groups. Further the role of multiple risk factors on training outcomes is worth of analyzing. The barriers in implementing the outpatient training program is required to be addressed to maximize patient participation in our Indian setup.

CONCLUSION

The tailor made program results in significant improvement in institution setup than self-monitored exercises, even though both the methods of training were having an impact to improve the functional capacity as measured by the SMW distance.

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ARBOVIRUSES OF IMPORTANCE IN INDIA: PART I. BIOLOGICAL, TRANSMISSION CHARACTERISTICS, CERTAIN UNIQUE ISSUES AND CLINICAL FEATURE

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ABSTRACT

There are over 100 viruses which are transmitted to humans by biological vectors of the insect group like mosquitoes, ticks and sandflies, these are called Arboviuses. The viruses usually have a single stranded RNA genome which could be negative stranded acting a template for both mRNA and replicative forms of RNA for virus maturation or may function as mRNA directly (positive strand) to cause the production of virus coded enzymes and then make replicative forms of RNA. Generally they are enveloped with varying capsid (shell) symmetry. Some of these viruses are endemic to tropical areas and presently due to global warming, vector spread to formerly uninfested areas and deforestation have caused epidemics in previously infection/disease free areas of the globe. They are predominantly zoonotic infections and are now a significant part of the newly emerging and reemerging infections. In India there are six important arboviral infections with at least two more emergent ones. These include dengue fever, West Nile fever, Japanese encephalitis, Chikungunya fever, Kayasanur forest disease, febrile illness agent the Sindbis virus, Chandipura virus and now the Crimean Congo hemorrhagic fever virus. The diseases range from uncomplicated fever, exanthematous febrile illness, and hemorrhagic fever leading to shock and encephalitis with some unusual complications as well.

***Key words** : Biological characteristics, Chandipura virus, Chikungunya virus, Dengue virus, epidemiology, Japanese encephalitis virus, Sindbis virus, Transmission, West Nile virus.*

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INTRODUCTION

Arboviruses (arthropod-borne viruses) are a large group of viruses that are spread by certain invertebrate animals (arthropods) serving as biological vectors. The vectors include: blood-sucking insects. They are most commonly spread by mosquitoes, ticks or members of the sandfly group.[1] Humans are often the only reservoir for some while others have an amplification host among species of birds and animals. These are often the source of infection for mosquitoes and other vectors. The infection is transmitted to the human host by bite of the infected arthropods. Most people infected with Arboviruses have few or no symptoms, but these agents can cause serious and potentially fatal inflammation of the brain (encephalitis) as well as other complications. Arboviruses can cause four types of illness: 1. Central nervous system illnesses, ranging in seriousness from mild viral meningitis to encephalitis, with coma, paralysis, and death. 2. Mild febrile illnesses, with or without rash 3. Hemorrhagic fevers that can be serious and life-threatening 4. Arthritis and rash, with or without fever.

More than 100 Arboviruses are known to cause disease in humans. Every major infection seen in the world caused by these viruses is an emerging or reemerging infection as per WHO definition. Most of these are classified into groups, or families. Salient features of Arboviruses seen in India are shown below in Table-1.

In India there are six important Arboviral infections with at least two more emergent ones. These include dengue fever, West Nile fever, Japanese encephalitis, Chikungunya fever, Kayasanur forest disease, febrile illness agent the Sindbis virus, Chandipura virus infection and now the Crimean Congo hemorrhagic fever virus.[2] (Table-2)

Biological features: DENV is an RNA virus of the family Flaviviridae; genus Flavivirus. The other members of the same genus Flavivirus include yellow fever virus, West Nile virus, St.Louis encephalitis virus, Japanese encephalitis virus, Kyasanur forest disease virus and Omsk hemorrhagic fever virus. All are enveloped RNA gene containing viruses.

The dengue virus (DENV) has a single stranded RNA genome which contains about 11,000 nucleotide bases. The genome codes for the three different types of protein molecules (C, prM and E) that form the virus particle and seven other types of protein molecules (NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5) that are only found in infected host cells. These protein molecules are required for replication of the virus. There are four strains of the virus, which are called serotypes, and these are referred to as DENV-1, DENV-2, DENV-3 and DENV-4. All four serotypes can cause disease. Infection with one serotype produces life long immunity to that sero type but only short term protection against the other serotypes.[3] Severe complications on secondary infection occurs particularly if someone previously exposed to serotype DENV-1 then contracts serotype DENV-2 or serotype DENV-3, or if someone previously exposed to type DENV-3 then acquires DENV-2.

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Table 1: Arboviruses seen in India: Table of Family host and diseases caused

Virus Family	Virus Genera	Virus Species	Vectors (Genus and species)	Diseases caused
Flaviviridae	Flavivirus (DENV)	Dengue virus	Mosquito (Aedes. aegypti)	Fever, hemorrhagic fever, shock syndrome[3,30,31]
Togaviridae	Alphavirus (CHIKV)	Chikungunya virus	Mosquito (Aedes aegypti)	Fever with arthralgia/ arthritis, encephalitis[32,33]
Flaviviridae	Flavivirus	Japanese encephalitis virus (JEV)	Mosquito (*Culx tritaeniorhynchus* and *Culex vishnui*)	Encephalitis[11,12,26,27]
Flaviviridae	Flavivirus (WNV)	West Nile virus	Mosquito (Culex pipiens, Culex tarsalis, Culex quinquefasciatus)	Encephalitis [13,17,28]
Rhabdoviridae	Vesiculovirus (CHPV)	Chandipura virus	Sandfly (Sergentomyia)	Encephalitis[38,39,40]
Bunyaviridae	Nairovirus	Crimean Congo Hemorrhagic fever virus (CCHFV)	Tick (Hyalomma detritum)	Hemorrhagic fever[20]
Flaviviridae	Flavivirus	Kyasanur Forest Disease virus (KFDV)	Tick (Haemaphysalis spinigera)	Encephalitis with haemorrhagic manifestation[22,37]
Togaviridae	Alphavirus	Sindbis virus (SINV)	Mosquitoes (Culex spp)	Fever with arthralgia and rash

Chikungunya virus (CHIKV) is a single-stranded RNA virus that belongs to the family Togaviridae, genus Alphavirus. This virus is transmitted only by mosquitoes. The Alphavirus group comprises 28 viruses, six of which can cause human joint disorders namely Chikungunya virus, O' nyong- On yong virus (central Africa), Ross River and Barmah Forest viruses (Australia and the Pacific), Sindbis virus (cosmopolitan), and Mayaro virus (South America, French Guyana).[4]

Japanese encephalitis virus (JEV) was previously known as Japanese B encephalitis, belongs to the family Flaviviridae. The reservoirs of JE are domestic pigs and wild birds. Transmission to humans may cause severe symptoms. Culex tritaeniorhynchus and Culex vishnui are the most important vectors of this disease. This disease is most prevalent in Southeast Asia and the Far East. JE virus belongs to genus flavivirus and is closely related to the West Nile virus and St Louis encephalitis virus. The positive sense single stranded RNA genome is packaged in the capsid which is formed by the capsid protein. The outer envelope is formed by envelope (E) protein and it is the protective antigen. It helps in entry of the virus to the inside of the cell. The genome also encodes a number of nonstructural proteins like (NS1, NS2a, NS2b, NS3, N4a, NS4b, and NS5). Based on the envelope gene (E) there are five genotypes (I - V). The Muar strain, isolated from patient in Malaya in 1952, is the prototype strain of genotype V. Genotype IV appears to be the ancestral strain and the virus appears to have evolved in the Indonesian-Malayasian region.[5]

West Nile virus (WNV) is a mosquito borne zoonotic arbovirus which belongs to the family Flaviviridae and genus Flavivirus. West Nile Virus is one members of the of the JE antigenic serocomplex of viruses. The genetic material of WNV is a positive-sense, single strand of RNA, which contains between 11,000 and 12,000 nucleotides long; these genes encode seven nonstructural proteins and three structural proteins. Based on the nucleotide sequence data of WNV there are four distinct lineages. Lineage I is associated with major epidemic. Indian isolates are classified under one of clades of Lineage 1(Lineage Ic) Lineage. Recently published study done with Indian strains over a period of 27 years indicates that these viruses clustered into a distinct lineage 2. [6,7,8]

Chandipura virus belongs to Rhabdoviridae family that is associated with encephalitis in humans. Chandipura virus is an enveloped RNA virus with an approximate genome length of 11,000 nucleotides. Viral genome codes for five polypeptides, namely, Nucleocapsid protein N, Phosphoprotein P, Matrix protein M, Glycoprotein G and Large protein L in five monocistronic mRNAs. The N protein encapsidates the genomic RNA into a nuclease resistant to protect it from cellular RNAse function. L and P protein together forms viral RNA dependent RNA polymerase for RNA polymerization. It was first identified in 1965 after isolation from the blood of two patients from Chandipura village in Maharashtra state, India.[9]

CCHF agent is a RNA virus belonging to the family Bunyaviridae genus Nairovirus. It is a widespread tick borne viral disease; a zoonosis that affects domestic animals, wild animals and humans. The pathogenic virus is common in East and West Africa. The genome is a circular, ambisense RNA in three parts - Small (S), Middle (M) and Large (L). The L segment is 11-14.400 in length while the M and S segements are 4.4-6.300 and 1.7-2.100 long respectively. The L segment encodes the RNA polymerase; the M segment encodes the

Table 2: EPIDEMIOLOGICAL TIMELINES OF CERTAIN ARBOVIRUSES IN INDIA

Year	Dengue Virus	Chikungunya Virus	Japanese Encephalitis Virus	West Nile Virus	Chandipura Virus
1952	-	-	-	Bombay[51]	-
1954	-	-	-	-	Madhya Pradesh
1955	-	-	Vellore[51]	-	-
1958	-	-	-	-	Maharashtra[41]
1963	-	West Bengal[68]	-	-	-
1964	Vellore	Tamil Nadu & Tamil Nadu[33]	- Pondicherry[33]	- -	- -
1965	-	Andhra Pradesh[33]	-	-	Maharashtra[41]
1966	Vellore, Tamil Nadu[43]	-	-	-	-
1967		-	-	South India[34]	- -
1968 to 1970	Tamil Nadu & Uttar Pradesh[30,33]	-	-	-	-
1973		Maharashtra[65]	-	-	-
1976	-	-	West Bengal[72]	-	-
1983	West Bengal[31]	Maharashtra[66]	-	-	-
1985	Rajasthan [43]	-	-	-	-
1988	Delhi, Gujarat [44]	-	Uttar Pradesh[74]	-	-
1989	-	-	Assam Kerala[72,73]	-	-
1990	West Bengal[45]	-	-	-	-
1993	Karnataka [46]	-	-	-	-
1996	Punjab, Uttar Pradesh,	- Haryana[48,49]	-	-	-
1997	Delhi[50]	-	-	-	Andhra Pradesh
2000	-	Maharashtra[66]	-	-	-
1999	-	-	Andhra Pradesh[40]	-	-
2000	-	-	Assam[71]	-	-
2001	Tamil Nadu,	- Madhya Pradesh[63,52]	Assam[71]	-	-
2002	-	-	Assam[71]	-	-
2003	Delhi, Madhya Pradesh, Tamil Nadu [63]	-	-	Andhra Pradesh, Tamil Nadu	Andhra Pradesh[40]
2004	-	-	-	Gujarat[51]	Gujarat[40]
2005	West Bengal [64]	AndraPradesh,	Uttapradesh[71] Karnataka, Maharastra, Rajastan, Gujarath, Tamil Nadu, Orissa and Kerala [66]	-	-
2006	Delhi [52,53]	AndraPradesh, Karnataka,	Assam Maharastra,Rajastan, Gujarath,Tamil Nadu, Orissa and [66,67]	Assam (Siraj et.al 2011) http://www.nrhmassam.in/ nvbdcp.php	
2007	Delhi ,Tamil Nadu, Andhra Pradesh [54,55,56,57,51]	Kerala [69,66]	Assam http://www.nrhmassam.in/ nvbdcp.php	Maharashtra	Andhra Pradesh[39]
2008	Delhi ,Kerala [57,51,59,60,61]	Kerala [70, 66]	-	-	-
2009	Delhi ,Maharashtra [57,62]	Kerala[32]	-	Maharashtra	-
2010	Maharashtra [62]	-	-	-	-
2011	-	-	-	Kerala[35]	-

*Crimean Congo Hemorrhagic fever virus reported in 2011, Gujarat, Kyasanur Forest Disease virus in Karnataka and Kerala.(The Hindu)

#Sindbis virus has been recovered from Migratory birds in Maharashtra so far no human cases have been reported in Inhttp://www.thehindu.com/news/national/kerala/youth-gets-kyasanur-disease-in wayanad/article4712468.ecehttp://www.thehindu.com/news/national/tamil-nadu/state-records-most-dengue-cases-and-deaths-this-year/article4003294.ece

envelope proteins (Gc and Gn); and the S segment encodes the nucleocapsid protein. The envelope protein is initially translated as a glycoprotein precursor which is then cleaved into two smaller proteins. Based on the sequence data seven genotypes have been recognised: Africa 1 (Senegal), Africa 2 (Democratic Republic of the Congo and South Africa), Africa 3 (southern and western Africa), Europe 1 (Albania, Bulgaria, Kosovo, Russia and Turkey), Europe 2 (Greece), Asia 1 (the Middle East, Iran and Pakistan) and Asia 2 (China, Kazakhastan, Tajikistan and Uzbekistan).[10] KFDV belongs to the family Flaviviridae. It is a tick-borne viral hemorrhagic fever endemic in South Asia. KFDV belongs to Russian Spring Summer Encephalitis group, a member of family Flaviviridae. Virions are spherical particles and 45 nm in diameter.[11]

Sindbis Virus (SINV) belongs to the family Togaviridae in the alphavirus subfamily. The virus was first isolated in 1952 in Cairo, Egypt. The virus is transmitted by mosquitoes Culex spp. Sindbis viruses are enveloped particles with an icosahedral capsid. Its genome is a single stranded RNA approximately 11.000 long. It has a 5' cap and 3' polyadenylated tail and therefore serves directly as messenger RNA in a host cell.[12]

Transmission characteristics and certain unique issues: The DENV is contracted from the bite of a striped Aedes Aegypti mosquito that has previously bitten an infected person. The mosquito flourishes during rainy seasons but can breed in water-filled flower pots, plastic containers, and cans year-round. One infected mosquito bite can cause the disease. The virus is not directly contagious to humans and cannot be spread directly from person to person. There must be a person-to-mosquito-to-another-person pathway.

The vector mosquitoes usually live between the latitudes of 35° North and 35° South below an elevation of 1,000 metres (3,300 ft) and bite primarily during the day. Other Aedes species that could transmit the disease include A. albopictus, A. polynesiensis and A. scutellaris. Humans are the primary host of the virus, but could also circulate in non human primates in a sylvatic form as seen in the Philippines.[29] A female mosquito that takes a blood meal from a person infected with dengue fever becomes itself infected with the virus in the cells lining its gut About 8–10 days later, the virus spreads to other tissues including the mosquito's salivary glands and is subsequently released into its saliva. The virus seems to have no detrimental effect on the mosquito, which remains infected for life. Aedes aegypti prefers to lay its eggs in artificial water containers, to live in close proximity to humans, and to feed off people rather than other vertebrates.[13]

The CHIKV is transmitted from human to human by the bites of infected female mosquitoes. Most commonly, the mosquitoes involved are Aedes aegypti and Aedes albopictus, two species which can also transmit other mosquito-borne viruses, including dengue. These mosquitoes transmit this virus with very similar dynamics to that of DENV.[14]

In a zoonotic cycle, JEV is transmitted by mosquito vectors between wild and domestic birds and pigs. Mosquitoes are vectors as well as a crucial intermediate replicative host for the normal enzootic cycle through birds and pigs. Both pigs and birds like heron, ducks, chicks etc. support high viremia and serve as the primary host for virus. Pigs are amplifying hosts with no evident signs of infection. After acquiring the infection from viremic pigs and completing the extrinsic cycle of 14 days, mosquito becomes infectious. In these natural amplifying hosts, however; virus does not produce encephalitis, although abortion occurs in pregnant sows. Virus is transmitted to humans by the bite of infected mosquito, which serves as a dead end host due to its short duration and low viremia in man. Humans are the incidental host and not the natural host for JEV infections.[15]

The WNV is transmitted through female mosquitoes, which are the prime vectors of the virus. The birds develop sufficient viral levels after being infected, to transmit the infection to other biting mosquitoes which in turn go on to infect other birds. In mammals, the virus does not multiply as readily (i.e. does not develop high viremia during infection), and mosquitoes biting infected mammals are not believed to ingest sufficient virus to become infected, making mammals so-called dead-end infections. Direct human-to-human transmission is believed to be caused only by occupational exposure or conjunctival exposure to infected blood.[16] The possible role of ardeid birds in maintenence of WNV has been described in India during inter-enzootic periods. Various zoophilic Culex species and Aedes albopictus mosquitoes can act as possible vectors for WNV transmission in different geographical regions. Transovarian transmission of virus has been experimentally demonstrated in the Culex vishnui species. By 2003 transmission of WNV through blood transfusion and organ transplant was confirmed.[17] Screening of blood donations for WNV RNA has been implemented in the United States.

CHPV has been isolated from sandflies in India and West Africa and is probably spread through its bite. The presence of the virus in Africa indicates a wide distribution although no human cases have been observed outside of India.[18]

CCHFV usually circulates between asymptomatic animals and ticks in an enzootic cycle. This virus has been found in at least 31 species of ticks, including seven genera of the family Ixodidae (hard ticks). Members of the genus Hyalomma seem to be the principal vectors. Transovarial, transstadial i.e. venereal transmission occurs in this genus. Hyalomma marginatum marginatum is particularly important as a vector in Europe, but CCHFV is also found in Hyalomma anatolicum anatolicum and other Hyalomma spp. Many

species of mammals can transmit CCHFV to ticks when they are viremic. Small vertebrates such as hares and hedgehogs, which are infested by immature ticks, may be particularly important as amplifying hosts. With a few exceptions, birds seem to be refractory to infection; however, they may act as mechanical vectors by transporting infected ticks. Migratory birds might spread the virus between distant geographic areas. Humans become infected through the skin and by ingestion. Aerosol transmission was suspected in a few cases in Russia. Sources of exposure include being bitten by a tick, crushing an infected tick on the bare skin, contacting animal blood or tissues and drinking unpasteurized milk. Human-to-human transmission occurs, particularly when skin or mucous membranes are exposed to blood during hemorrhages or tissues during surgery. Possible horizontal transmission has been reported from a mother to her child.[19,20,21]

The main hosts of KFD virus are small rodents, shrews, bats but monkeys may also carry the virus. KFD virus is transmitted from the bite of an infected tick (Haemaphysalis spinigera is the major vector). Humans can get these diseases from a tick bite or by contact with an infected animal, such as sick or recently dead monkey. Larger animals such as goats, cows, and sheep may become infected with KFD, but they do not have a role in the transmission of the disease. Furthermore, there is no evidence of the disease being transmitted via the unpasteurized milk of any of these animals. Sindbis virus is maintained in nature by transmission between vertebrate (bird) hosts and invertebrate (mosquito) vectors. Humans are infected with Sindbis virus when bitten by an infected mosquito.[22,23]

Clinical features: Typically, people infected with dengue virus are asymptomatic (80%) or only have mild symptoms such as an uncomplicated fever. Others have more severe illness (5%), and in a small proportion it is life-threatening. The incubation period (time between exposure and onset of symptoms) ranges from 3–14 days, but most often it is 4–7 days. Therefore, travelers returning from endemic areas are unlikely to have dengue if fever or other symptoms start more than 14 days after arriving home. Children often experience symptoms similar to those of the common cold and gastroenteritis (vomiting and diarrhea), but are more susceptible to the severe complications. Symptoms range from a mild fever, to incapacitating high fever, with severe headache, pain behind the eyes, muscle and joint pain, and rash. In children, DF is usually mild. In some adults, DF may be the classic incapacitating disease with severe bone pain and recovery may be associated with prolonged fatigue and depression.[24]

Dengue haemorrhagic fever (DHF): Patients present with fever, abdominal pain, vomiting, bleeding and is a potentially lethal complication, affecting mainly children. Early clinical diagnosis and careful clinical management by experienced physicians and nurses increase survival of patients. Dengue Shock Syndrome (DSS): This may develop as a complication of DHF. DHF: Is evidenced by one or more of the following: A positive tourniquet test, petechiae, ecchymosis or purpura, bleeding from mucosa (mostly epistaxis or bleeding from gums), injection sites or other sites, haematemesis or melena, a combination of these features along with evidence of plasma leak are seen[3,30,31]

Symptoms of Chikungunya includes fever, debilitating arthralgia (joint pain), swelling of joints, stiffness of joints, myalgia (muscular pain), headache, fatigue (weakness), nausea, vomiting and rash.[32] The incubation period (time from infection to illness) can be 2-12 days, but is usually 3-7 days.[33] "Silent" CHIKV infections (infections without illness) do occur; but how commonly this happens is not yet known. Acute Chikungunya fever typically lasts a few days to a couple of weeks, but some patients have prolonged fatigue lasting several weeks. Additionally, some patients are reported with incapacitating joint pain, or arthritis which may last for weeks or months. No deaths, neuro-invasive cases, or hemorrhagic cases related to CHIKV infection have been conclusively documented in the scientific literature. CHIKV (RNA and proteins) have been found in perivascular synovial macrophages in one chronic patient 18 months post-infection. CHIKV persists in the synovial tissue was associated with fibroblast hyperplasia and strong angiogenesis, leading to cell apoptosis (identification of cleaved poly [ADP-ribose]-positive polymerase cells) and tissue lesions as evidenced by high levels of matrix metalloproteinase. These observed cellular and molecular events may contribute to chronic arthralgia /arthritis targeted by methotrexate used empirically for effective treatment, but with immunosuppressive function in the context of viral persistence . Infection is whether clinical or silent is thought to confer life-long immunity.[25]

There is an incubation period of 4-14 days in humans during JEV infection and patients present with few days of fever including coryza, diarrhea and rigors. Headache, vomiting and reduced levels of consciousness is followed by convulsions. Spontaneous recovery is observed in a large proportion of patients, termed as abortive encephalitis. In some patients, aseptic meningitis without encephalopathic features may be seen.[34] Convulsions occur more frequently in children in upto 85% cases than in adult patients [27] Complete recovery occurs in mild cases, severe cases improve but patients especially may have neurological deficits. Severe symptoms include high fever, headache, neck stiffness, confusion, tremors and convulsions.[34,27]

Infection wih WNV can result in encephalitis or meningitis blocking the flow of blood to the brain. This could lead to coma, paralysis, or even death. The neurological effects may be permanent in some people. This virus causes symptoms of fever, similar to those found in flu and causes acute encephalitis. The disease progresses rapidly from influenza-like symptoms to coma resulting in death with a fatality rate is as high as 58%. Incubation period of WNV is 2 to 14 days, 80% of the patients are asymptomatic

or with mild symptoms, 20% have a Dengue like illness ranging from mild to severe and less than 1% have neuroinvasive disease like meningitis, encephalitis or poliomyelitis-like flaccid paralysis. Neurological disease is common in elderly and immunocompromised patients.[28] About 50% of these people can have persistent sequelae for more than 12 months after the infection. Neurological manifestations can be altered sensorium, fatigue, neck stiffness, movement disorders, tremors, polyradiculopathy. Children experience milder illness than adult. Ophthalmic manifestations like chorioretinitis, optic atrophy, optic neuritis have been reported frequently but the incidence has not been determined so far.[28,36]

The onset of CCHF is seen typically, after a 1–3 day incubation period following a tick bite (5–6 days after exposure to infected blood or tissues) and flu-like symptoms appear, which may resolve after one week. In up to 75% of cases signs of hemorrhage appear within 3–5 days of the onset of illness. Initial symptoms are mood instability followed by agitation, mental confusion , petechiae on throat, nosebleeds, bloody urine, vomiting, and black stools. Patients may present with acute painful hepatomegaly with jaundice, acute renal failure or disseminated intravascular coagulation resulting in shock and acute respiratory distress. Patients usually begin to show signs of recovery after 9–10 days from the onset of symptoms, however 30% of the cases result in death on the second week of the illness. As the illness progresses, large areas of severe bruising, severe nosebleeds, and uncontrolled bleeding at injection sites can be seen, beginning on about the fourth day of illness and lasting for about two weeks.[20]

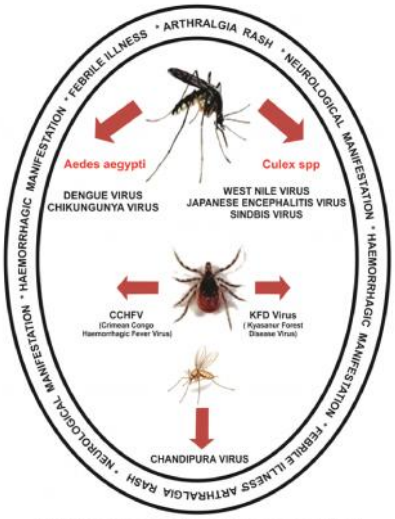

Fig.1: Schematic representation of virus, vector and clinical manifestation of Arbovirus seen in India

After an incubation period of 3-8 days, the symptoms of KFD begin suddenly with fever, headache, severe muscle pain, cough, dehydration, gastrointestinal symptoms and bleeding problems. Patients may experience abnormally low blood pressure, and low platelet, red blood cell, and white blood cell counts. After 1-2 weeks of symptoms, some patients recover without complication. However, in most patients, the illness is biphasic and the patients begin experiencing a second wave of symptoms at the beginning of the third week.[22,37] These symptoms include fever and signs of encephalitis. Infection is characterized by coincident onset of fever with rash. Arthritic pain, with headaches, general weakness or malaise are also common. Rash lasts around 10 days, but quite often no clinical disease manifestations are recognized. A schematic representation of the vector and its relationship to the virus and clinical features in the host is shown in Figure 1.

CONCLUSIONS

India has eight established Arboviral diseases. Of these, five are very important and which have caused epidemics in wide parts of the country. Among these viruses with epidemic potential dengue virus and JE virus produce periodic epidemics. There are three serious hemorrhagic fevers with fatal complications reported in different parts of India. These include the KFD and CCHF which has so far been reported in geographically circumscribed areas only. DHF has been reported from multiple places of the country. It could be said that Arboviral infections contribute to significant amount of morbidity due to infectious diseases in India.

....(To be contd. in Part II)

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A RARE CAUSE OF NEONATAL CEREBRAL ABSCESS

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ABSTRACT

This is the first case report of neonatal sepsis with Streptococcus pseudopneumoniae. This preterm baby with Intrauterine Growth Restriction (IUGR) developed brain abscess on day 6 of life and the organism both in blood and cerebrospinal fluid (CSF) could be identified by genomic studies and not by blood culture. Streptococcus pseudopneumoniae is usually a respiratory pathogen in chronic obstructive pulmonary disease (COPD) patients whereas in the present scenario it caused cerebral abscess in a neonate which makes this an unusual presentation.

Keywords: *Brain abscess Neonatal sepsis, Streptococcus pseudopneumoniae.*

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INTRODUCTION

Streptococcus pseudopneumoniae was first described in 2004, isolated from lower respiratory tract secretion.[1] It is similar to Streptococcus pneumoniae except for few microbiological and genomic characteristics. Streptococcus pneumoniae is known to cause neonatal sepsis[2] whereas *Streptococcus pseudopneumoniae* is being increasingly reported in adults with chronic obstructive pulmonary disease (COPD).[3] We describe the clinical course of a newborn baby with cerebral abscess caused by *Streptococcus pseudopneumoniae*. This is the first case report of neonatal *Streptococcus pseudopneumoniae* sepsis in the literature.

CASE REPORT

A preterm baby of 34 weeks of gestational age and birth weight of 1390 grams was born to mother with gestational hypertension. Antenatal scans revealed evidence of intrauterine growth restriction with abnormal umbilical arterial doppler flow and oligohydramnios. The baby was born by caesarian section, vigorous at birth and admitted in neonatal intensive care for preterm care. Bedside neurosonography performed on day 1 of life was normal. On day 6, baby developed tachycardia and then apnea needing mechanical ventilation. Subsequently, baby developed features of shock needing inotropic support. The baby was also neurologically depressed and had seizures. Investigations revealed high C-Reactive Protein (CRP) (8.56 mg/dL), leucocytosis and cerebrospinal fluid (CSF) pleocytosis and hypoglycorrhacia. Two blood cultures and a CSF culture were sterile. Magnetic resonance imaging (MRI) done following an abnormal bedside neurosonography showed multiple large cavitating lesions with areas of hemorrhage predominantly involving frontal and parietal lobe with mild perilesional edema and few of the lesions showing peripheral enhancement implying liquefactive necrosis with secondary abscess formation, features suggestive of citrobacter infection (Figure 1-4). Molecular diagnosis using 16S rRibonucleic acid (rRNA) polymerase chain reaction (PCR) and gene sequencing with PCR (ABI Prism 310/3100 AVANT genetic analyzer) revealed *S.pseudopneumoniae* in both blood and CSF. Maternal nasopharyngeal swab culture was negative. Baby was treated with antibiotics for 6 weeks. MRI of the brain at 8th week of life showed communicating hydrocephalus with thinning of bilateral cerebral parenchyma and features were suggestive of post meningitic sequelae. Neurosurgical opinion was obtained. In view of the high CSF protein content, ventriculo-

FIGURES: MRI Images showing cerebral abscess

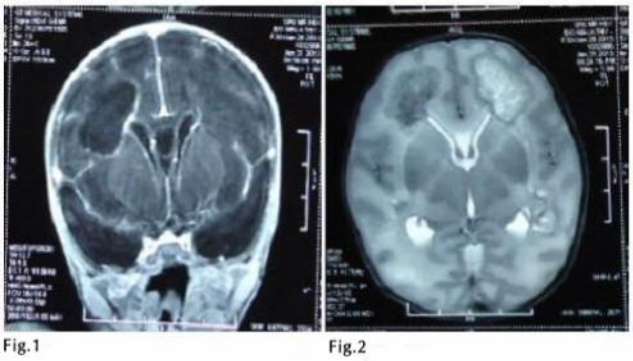

Fig.1 Fig.2

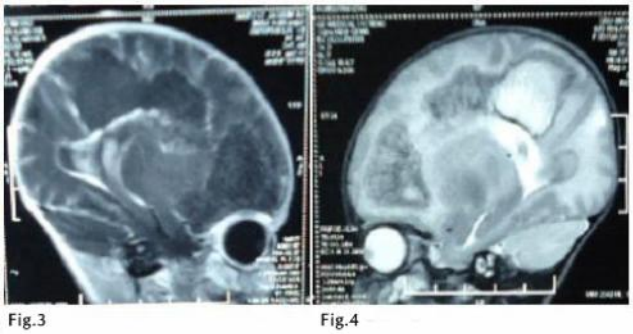

Fig.3 Fig.4

Fig.1: Sagittal view of T1 image showing large abscess in frontal and parietal area

Fig.2: Sagittal view of T2 image

Fig.3: Coronal view of T1 image

Fig.4: Coronal view of T2 image

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peritoneal shunt was deferred. Serial ventricular taps were done. Baby was thriving well but had abnormal tone during Hammersmith Neonatal Neurological Examination. Baby was discharged with regular follow up plan and subsequently had ventriculoperitoneal shunt.

DISCUSSION

This is the first case report of neonatal sepsis with *Streptococcus pseudopneumoniae* (*S. pseudopneumoniae) S.pseudopneumoniae* is a unique organism which belongs to Streptococcus mitis/oralis group of viridans Streptococci and shares some characteristics with S. pneumoniae. The key differentiating features are insolubility in bile, absence of a pneumococcal capsule, resistance or indeterminate susceptibility to optochin when incubated in 5% carbon dioxide (CO_2) and susceptibility to optochin when incubated in ambient air. Sometimes this organism is termed as atypical Streptococci.[4] As far as India is concerned Mohammadi et al[3] have reported S.*pseudopneumoniae* as an emerging respiratory pathogen. Although this organism is reported to be a respiratory pathogen, in the present case it caused neonatal cerebral abscess, which makes this case report unique.

Though *S.pseudopneumoniae* can be cultured using the routine bacteriological methods, in our case, blood and CSF culture were sterile. The organism was identified by genomic sequencing of 16S rRNA using conventional PCR. 16S rRNA is universally present in nearly all bacteria and not in humans. 16S rRNA gene sequence information has an expanding role in the identification of bacteria in clinical settings.The reasons include (i) its presence in almost all bacteria, often existing as a multigene family, or operons; (ii) the function of the 16S rRNA gene over time has not changed, suggesting that random sequence changes are a more accurate measure of time (evolution); and (iii) the 16S rRNA gene (1,500 bp) is large enough for species level bacterial identification.[5] 16S rRNA can be identified using molecular assays like PCR. Sequencing of the 16S rRNA and its deoxyribonucleic acid (DNA) homology with the Genbank sequences would help in identification of the bacterial species. Conventional PCR as a diagnostic tool for neonatal sepsis is being used as an add-on test to identify the organism. [6] In a meta-analysis of molecular assays in the diagnosis of neonatal sepsis done by Pammi et al, the mean sensitivity and specificity were 90% and 96% respectively. The main disadvantage of the diagnostic technique is the inability to get susceptibility pattern to antibiotics as well as inability to differentiate colonizers from pathogens when applied to non sterile sites.

Early onset neonatal sepsis (EOS) occurs within 7 days of life and is acquired usually by vertical transmission from the mother.[7] The present scenario could be a possible early onset sepsis. As carrier detection is better with nasopharyngeal swab, the same was done for the mother but it was negative. Mother was asymptomatic. Nosocomial infection of S.*pseudopneumoniae* has not been reported and it is usually a community acquired lower respiratory tract pathogen in adults with COPD.[3]

Screening neurosonography done on day 1 of life to detect intraventricular haemorrhage was normal and the subsequent scan on day 6 done for seizures showed multiple abscesses. This underscores the importance of bedside ultrasonography. In the present case scenario, *S. pseudopneumoniae* caused neonatal sepsis with multiple brain abscesses and ventriculitis. Gram negative organism especially Citrobacter species have been implicated in neonatal brain abscess more often even though isolated cases of group B Streptococci and staphylococcus have been reported [8].The major pathological feature of *Citrobacter koseri* meninigitis is vasculitis, followed by infarction, necrosis and liquefaction of large regions of white matter.[9,10] MRI of the brain in this neonate also revealed features of liquefactive necrosis with secondary brain abscess which closely resembled gram negative infection.

Meropenem was started for possible Citrobacter infection. Arbique et al in 2004 found *S. pseudopneumoniae* species to be susceptible to Penicillins,[1] but later Rolo D et al in 2013 reported that around 60% were not susceptible to Penicillins.[11] In the present case, antibiogram were not available with molecular diagnosis. As there was clinical improvement with Meropenem, the same was continued for treatment.

CONCLUSION

Streptococcus pseudopneumoniae a member of the Viridans Streptococci has been isolated from sputum specimens in patients with COPD[4] and from invasive and non-invasive diseases. To the best of our knowledge, this is the first case report of *S pseudopneumoniae* neonatal sepsis reported in literature. This article also brings the importance of bedside ultrasonography which helped in proceeding to further evaluation and the molecular diagnosis in neonatal sepsis which helped in identifying the organism.

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DUODENAL CARCINOID : A CASE REPORT

Soumya John, V.Bhaskar, R.Chandru, Ramya Ramakrishnan

ABSTRACT:

Duodenal carcinoid is a rare form of neuroendocrine tumor. It accounts for 2 % to 5 % of carcinoid tumor in the literature. In this article we present a case of a 55 yr old female who was diagnosed to have low grade well differentiated neuroendocrine tumor of the duonenum. Intraoperative endoscopic tattooing and excision of the lesion was done for the same.

Key words: *Carcinoid, Duodenum, Endoscopic tattooing, Neuroendocrine tumour.*

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INTRODUCTION

Carcinoid tumour is a rare neuroendocrine tumour that originates in the enterchromaffin cells lining the gastrointestinal tract, retroperitoneum and large bronchi. The term carcinoid tumour was coined by Siegfried Oberndorfer in 1907.[1] This was used to describe these small tumors that were mistaken for carcinomas initially. Carcinoids located in the duodenum are rare as it accounts for only 2 % to 5 % of all carcinoid tumors in the literature[2]. They exhibit a slow growth, lack endocrine activity, with a low metastatic potential.

CASE REPORT

A 55 year old female patient presented to the out patient department with occasional epigastric pain and history of dyspepsia for 6 months. Routine blood investigations and Ultrasound abdomen were essentially normal. Upper gastrointestinal endoscopy showed two polypoidal lesions of 1.5 cm each in D1(Fig 1) and they were biopsied. Histopathology revealed a carcinoid tumor. Immunohistochemistry was strongly positive for vimentin, synaptophysin and chromogranin. Contrast enhanced computed tomography was normal. The levels of 5 Hydroxyindoleacetic acid (5-HIAA) in 24 hrs urine was also normal (2-7mg/24hr).

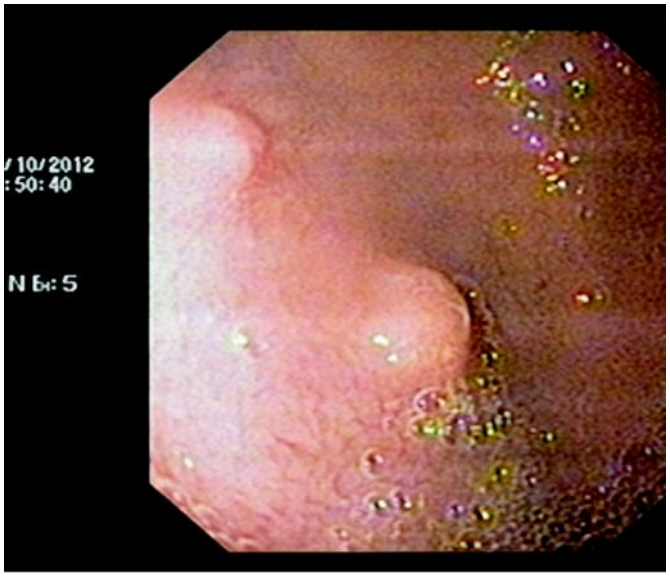

Fig 1. Upper gastrointestinal endoscopy

As there were two lesions a few centimetres apart, and their size were more than 1 cm, we decided to do open resection of the duodenum. Intraoperative localization of the tumour was done using endoscopic tattooing. By using endoscopy, the tumour site at duodenum was localised and India ink was injected for tattooing the area of tumour. Laparotomy was done and the tattooed area was identified at D1 (Fig 2). Resection of pylorus and first part of duodenum was done (Fig 3) with closure of duodenal stump and gastrojejunostomy. The specimen was sent for histopathology.

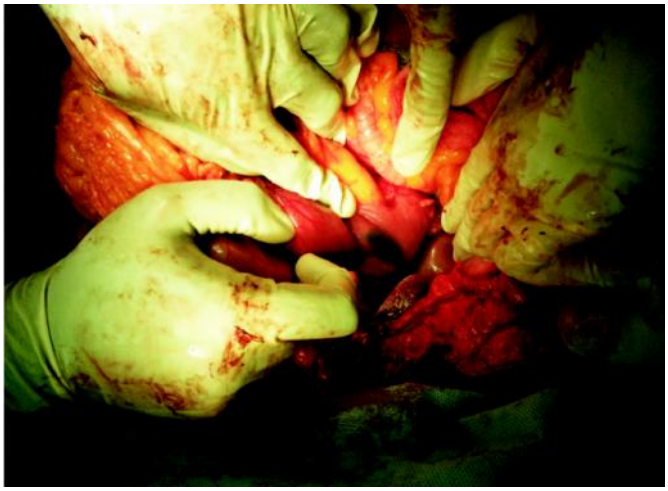

Fig 2. Identification of the tattooed area.

The postoperative course was uneventful. Patient was discharged on the 7th post operative day. The definitive histopathology of the tumour showed low grade well differentiated carcinoid tumour (Fig4). Patient was advised to come for a regular follow up with a repeat upper gastrointestinal endoscopy six months later.

DISCUSSION:

Carcinoid tumour arises from the Kulchitsky or enterochromaffin like cells of the intestinal crypts. To date, eight different neuroendocrine cell types have been identified and each cell type has characteristic neurosecretory granules, some with identified secretory products

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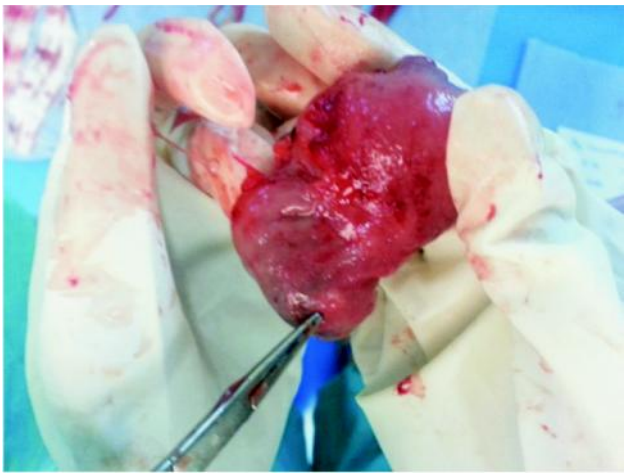

Fig 3. Resected specimen

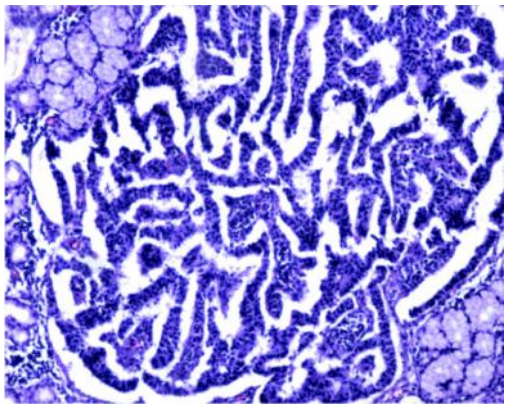

Fig 4. Histopathology showing low grade well differentiated neuroendocrine tumor.

for example : enterochromaffin cells secrete serotonin, enterochromaffin like cells secrete histamine, A cell secrete glucagon, D cells secrete somatostatin and G cells secrete gastrin. They are identified on histopathology by their affinity for silver salts and by their positive reaction to neuroendocrine markers such neuron specific enolase, synaptophysin and chromogranin.

The incidence of carcinoids in the population is very low,1 to 2.6 per 100 000 population.[3] Carcinoids form only 0.7 – 1.8% of all primary bowel neoplasms. Within the gastrointestinal tract, carcinoid tumors are most commonly found in the appendix followed by the ileum, rectum and stomach.[4] Carcinoid tumours of the duodenum are rare and constitute only 3.4 – 11.9% of all duodenal neoplasms.[5] Most of them are located in the first or second part of the duodenum.

Risk factors for small intestinal carcinoids include alcohol, female gender and positive family history. Our patient was a lady but was not an alcoholic and had no family history of similar tumors. Small intestinal carcinoids also occur with an increased frequency in a few inherited syndrome such as Multiple Endocrine Neoplasia1.

The World Health Organization classification of gastrointestinal endocrine tumours recognizes four main tumour categories[6] 1) well differentiated endocrine tumour, 2) well differentiated endocrine carcinoma (WDEC, malignant carcinoid) 3) poorly differentiated endocrine (small cell carcinoma) 4) mixed exocrine endocrine tumours.

Five major types of neuroendocrine tumours can be seen in the duodenum[7] 1) gastrinomas (type1) are the most common and are usually seen in the proximal duodenum. One third are associated with Zollinger Ellison Syndrome and Mutiple Endocrine Neoplasia1. 2) Second in frequency are somatostatinoma (type 2) which often have a periampullary location. They may be associated with Von Reckilinghausen disease. 3) gangliocytic paragangliomas (type 3) are benign tumours found at the ampulla or in the periampullary region. 4) Type 4 is rare and contains tumour that produce serotonin and calcitonin.5) Poorly differenciated endocrine carcinoma (Type 5) is extremely rare and highly malignant and is usually located at the ampulla of Vater.

Most duodenal carcinoids are asymptomatic and are detected during upper gastrointestinal endoscopy for unrelated symptoms such as abdominal pain (37%), upper gastrointestinal bleeding, anemia (21%), and jaundice (18%). In contrast, ampullary carcinoids more frequently cause symptoms, with up to 60% presenting with jaundice. Duodenal neuroendocrine tumors are usually hormonally silent (60-98%) as was our patient. Very few patients develop carcinoid syndrome characterised by diarrhea, flushing, carcinoid heart disease, bronchial constriction and abdominal pain. A life threatening exacerbation of carcinoid syndrome called carcinoid crisis can be triggered by anesthesia, drugs that causes release of histamine (6-18 Fluorodihydroxy phenylalanine) or surgery.

Diagnosis is usually made by upper gastrointestinal endoscopy and histological examination. Endoscopic ultrasound helps in detecting the size and depth of infiltration and also to assesses loco-regional lymph nodes. Serum and urine neuroendocrine markers (Chromogranin A and 5–Hydroxyindole acetic acid) may have diagnostic and prognostic values and used in follow up. Conventional imaging methods (Computerised tomography, Magnetic Resonance Imaging) can assist with tumour staging and localization. Octreoscan (using 111 In labelled octreotide) not only helps in detection, but also predicts therapeutics effects of somatostatin analogues in these tumours. Ga-DOTATOC–PET/CT is a Gallium composition used for functioning image of PET and it's a new, very sensitive method for visualising metastatic intestinal carcinoids. Due to frequent carcinoid heart disease (30 to 60%) every patient with carcinoid syndrome should have cardiac imaging.

In a study, Waisberg et al identified three pathologic features in the primary tumour as independent risk factors for metastasis: invasion of the muscularis propria, size > 2cm and the presence of mitotic figures.[8]

Treatment options include endoscopic resection, transduodenal excision, a wedge resection of the duodenum, segmental and distal duodenectomy and pancreaticoduodenectomy. Decision about surgery depends upon the location, size and stage of the tumour. Extensive loco-regional resection is meant for tumours more than 2 cm and also tumour of any size with lymph node metastasis. European guidelines recommended that duodenal carcinoids less than 10mm in diameter that are confined to the submucosa as seen on endoscopic ultrasound, should be treated by endoscopy in the absence of apparent lymph-node invasion and distant metastases.[9]

The surgical approach to duodenal carcinoid has been conventionally through laparotomy until recently, where a laparascopic approach (transduodenal excision of the tumour or wedge resection of the duodenum) was successfully done by Van de Walle.[10]

CONCLUSION:

Duodenal carcinoid is rare and presents with symptoms of gastritis or dyspepsia. Diagnosis is incidentally made by upper gastrointestinal endoscopy and histopathology. When diagnosed, although they are very small and innocuous, these tumours have to be resected due to their potential for metastases. With prompt treatment, the long term prognosis is good.

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CASE REPORT: A CASE OF PULMONARY NOCARDIOSIS

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ABSTRACT :

Nocardiosis is caused by nocardia species which are gram positive , rod shaped, partially acid fast bacteria occasionally detectable in environmental sources such as soil. Nocardiosis are usually seen in immunocompromised, most commonly in malignancies, acquired immunodeficiency syndrome (AIDS), organ transplantation, patients who are on steroids and pre existing pulmonary disease, rarely in healthy. In this case report, we present a 48 year old female with no co-morbidities presented with complaints of fever, cough with minimal expectoration for 2 weeks duration. Chest Roentogogram showed non homogenous opacities in left midzone and lowerzone. Sputum Acid Fast Bacilli (AFB) and gram stain done showed plenty of gram positive branching bacilli, suggestive of nocardia. Sputum culture was suggestive of Nocardiosis growth for which she was started on Cotrimoxazole.

Keywords: *AIDS, Cotrimoxazole, immuno-compromised, nocardiosis*

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INTRODUCTION:

Nocardiosis is a localized or disseminated infection caused by a soil-borne actinomycete[1]. Nocardia most commonly causes pneumonia, but also infects the central nervous system (CNS) and skin. Less commonly this organism causes disseminated infection. Pulmonary Nocardiosis is generally subacute in onset and is most commonly misdiagnosed as pulmonary tuberculosis. We describe a immunocompetent patient presenting with low grade fever, cough with minimal expectoration initially suspected to have tuberculosis, but finally turned out to be nocardiosis.

CASE REPORT :

A 48 yr old female without any comorbid illness presented with complaints of 2 weeks of fever, cough with minimal expectoration. On examination, fine crepitations heard over the left mammary and axillary areas associated with wheeze. Chest X ray done showed non homogenous opacities in left midzone and lowerzone regions (Fig.1). High resolution computed tomography (HRCT) thorax done showed patchy areas of consolidation involving the apical segment of right upper lobe, anterior and apicoposterior segment of left upper lobe, Inter lobar thickening with tree in bud nodules involving bilateral lung fields and subcentimeter lymphnodes in lower paratracheal and pretracheal regions – features suggestive of endobronchial spread (Fig.2). Her baseline investigations done were normal. Modified Ziehl-Neelsen staining of sputum done showed plenty of gram positive branching bacilli, consistent with the morphology of nocardia was seen (Fig.3). She was further evaluated to rule out immunocompromised status. Her past history was insignificant. Human Immuno deficiency Virus (HIV) -1 and 2 was negative. Diabetes was ruled out. Sputum culture, revealed *Nocardia asteroides* growth appearing as greyish white, dry, rough, raised and irregular colonies. She was started on cotrimoxazole [Trimethoprim (160 mg) + Sulfamethaxazole (800mg)] twice a day. Patient was followed up regularly and her breathlessness improved and

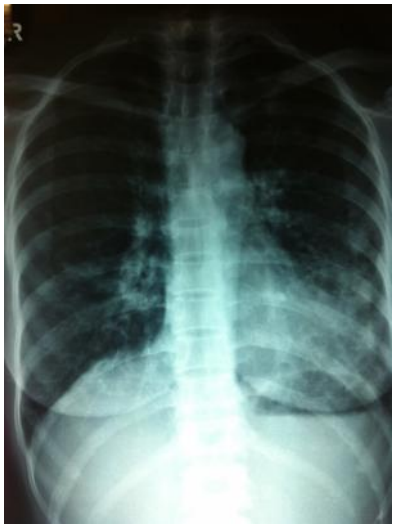

Fig.1: Left mid zone non-homogenous opacities

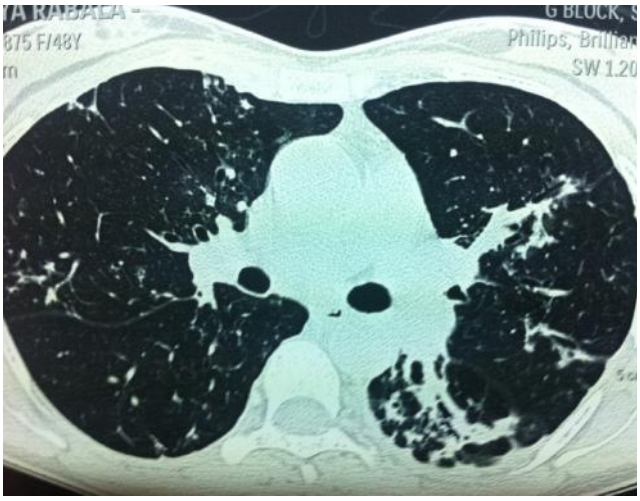

Fig.2: Patchy areas of consolidation in left apico-posterior lobe with inter-lobar septal thickening

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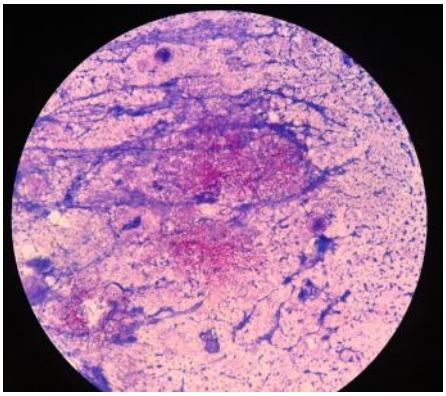

Fig.3: Gram positive filamentous bacteria showing branching pattern consistent with nocardia

she became afebrile from day 4 of treatment. The repeat chest x-ray done 2 weeks later, showed clearing of opacities.

DISCUSSION:

Pulmonary nocardiosis is a subacute or chronic pneumonia caused by actinomycetes of genus nocardia. It is an opportunistic, localized or disseminated granulomatous infection[2]. *Nocardia asteroides* is the most common pathogen responsible for the infection in humans, other species are *Nocardia brasiliensis* and *Nocardia otitidiscaviarum*.[3] Nocardia are branched gram-positive, variably acid-fast, strictly aerobic bacteria, and fragmentation occurs as it ages old resulting in rod-shaped or coccoid elements.[4]

Pulmonary infection is usually produced by *Nocardia asteroids*, whereas *Nocardia brasiliensis* causes cutaneous and subcutaneous abscesses.[3] Nocardiosis can be differentiated from actinomycosis by its lesser tendency to form sinus tract and a greater tendency for dissemination usually to the brain.[5] Nocardia have a substrate mycelium that fragments into bacillary or coccoid elements, whereas strains of streptomyces have nonseptate substrate mycelium and does not fragment. Grocott silver method is the most sensitive screening stain for detecting nocardiosis.[2] Paper chromatograms made from the whole-cell hydrolysates clearly demonstrates mesodiaminopimelic acid as a major constituent of cultures of Nocardia species, and LL-diaminopimelic acid in Streptomyces. Pulmonary nocardiosis is acquired by inhalation from contaminated soil.

Clinical manifestations include inflammatory endobronchial masses or localized or diffuse pneumonias.[6] Dominant symptoms are cough with sputum, fever. 40% of patients with disseminated nocardiosis have pulmonary infection; so predominantly presenting with pulmonary symptoms. Disseminated disease occurs in half of pulmonary nocardiosis cases.[7] Mortality is increased in disseminated disease, more commonly in adults.

Early nocardiosis present as localized broncho-pneumonia, and as the lesion progress, complete lobular consolidation may appear.[8,9] The most common findings include localized consolidation, cavitations, and lobar infiltrative disease with characteristically thick-walled cavities. Computed Tomography (CT) findings include consolidation with or without cavitation, multiple discrete pulmonary nodules, pleural effusion, and chest wall extension. Acquired immuno deficiency syndrome (AIDS) patients with pulmonary nocardiosis have more irregular, spiculated nodules, and a high incidence of cavity,[10] predominance of upper lobe involvement.

Sulfonamides have long been the first-line antimicrobial therapy for nocardiosis. Among the sulfonamides, sulfadiazine is generally preferred because of its CNS and cerebro spinal fluid (CSF) penetration. Although not convincingly demonstrated superior, trimethoprim-sulfamethoxazole (TMP-SMZ) is considered the therapy of choice.[11] TMP-SMZ dosages for adults with normal renal function are 2.5-10.0 mg/kg (TMP) and 12.5-50 mg/kg (SMZ) twice a day, according to the severity of the infection.[1] The efficacy of sulfonamides and their combination with TMP are maximal against *Nocardia brasiliensis,* most (>90%) *Nocardia asteroides* complex isolates, and *Nocardia transvalensis*.[12] Alternative therapies include carbapenems (imipenem or meropenem), third-generation cephalosporins (cefotaxime or ceftriaxone), and amikacin, alone or in combination. Linezolid and Tigecycline in vitro activity and in vivo efficacy has been reported.[11,13]

The duration of treatment in immunocompetent patients with pulmonary or systemic nocardiosis should be treated for a minimum of 6months. Patients with CNS infection and immunosuppressed should be treated for 12 months.[14]

CONCLUSION:

Pulmonary and systemic infection with nocardiosis has been documented in both normal and compromised hosts.[1] Our patient was neither immunosuppressed nor on steroids but she was a female working in fields with continous exposure to soil and polluted water which could have predisposed her to Nocardia infection. Clinical or radiographic presentation of pulmonary nocardiosis is not sufficiently distinctive to be diagnostic. Clinical manifestations vary between patients and are not usually specific. There is a need to consider pulmonary nocardiosis in the differential diagnosis of tuberculosis since both conditions mimic each other clinically. It is important to perform modified acid fast staining to rule out a diagnosis of nocardia when gram positive branching filamentous bacteria are seen on gram stain.

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STRONGYLOIDES - A TRESPASSER IN THE STOMACH

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INTRODUCTION

Strongyloides stercoralis, an intestinal nematode of humans is endemic in tropical, sub tropical and temperate climates.[1] The infective form is the filariform larvae that penetrates the skin, then migrates to the lungs. Larvae then ascend the airway, are swallowed and mature in the mucosa of the small intestine, especially duodenum and jejunum. The eggs mature rapidly into rhabditiform larvae and are observed in the stool specimens. *Strongyloides stercoralis* may infect many organs in hyperinfection, but gastric involvement is very rare.

A 52 year old female patient presented to the out patient department of Medicine with a history of abdominal pain, nausea and vomiting of 15 days duration. She was on corticosteroid therapy for rheumatoid arthritis. She also carried a diagnosis of drug induced gastritis and esophageal candidiasis. Laboratory evaluation revealed neutrophilic leucocytosis, hyponatremia, hypochloremia and mild proteinuria. Rest of the parameters were within normal limits. The serological parameters HBsAg, HIV and anti HCV were all negative. Upper gastrointestinal endoscopy revealed whitish plaques in the esophagus. The gastric mucosa revealed linear streaks of erythema and erosions (Fig 1) involving the fundus, body and antrum. Samples were taken from the gastric body and antrum and sent for histopathology.

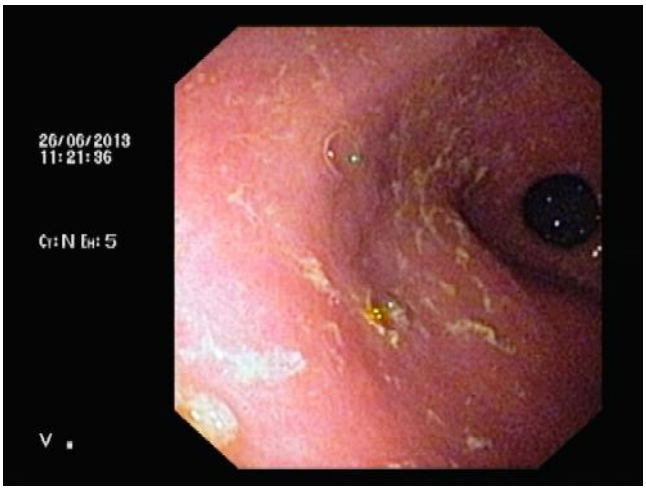

Fig.1: Endoscopy-Body of stomach showing linear streaks of erythema &erosions.

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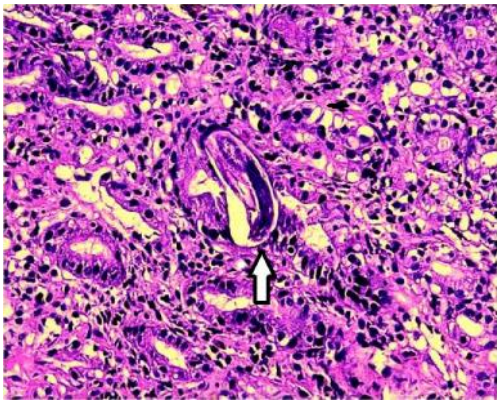

Fig.2: Microphotograph of gastric biopsy with larva of *Strongyloides stercoralis*(arrow) -H & E (X200)

Hematoxylin and eosin stained sections showed H.*pylori* associated chronic gastritis with moderate activity. The gastric mucosa also showed numerous cross section of rhabditiform larvae of *Strongyloides stercoralis* in the glands (Fig 2). There was also evidence of scattered eosinophils in the lamina propria possibly correlating with the intensity of infection. The patient was treated with anti-helminthic medications and requested to come for follow up. The patient received three drugs regimen for H.pylori consisting of T.Azithromycin, T.Metronidazole, T.Pantoprazole for two weeks.

DISCUSSION

Strongyloidiasis is a worldwide parasite infection, most densely distributed in areas characterized by high temperature, humidity and poor hygiene.[2,3] Gastrointestinal infection can be asymptomatic for years or present with nausea, vomiting, diarrhoea triggered by the use of H2 blockers, immunosuppressants, old age, malnutrition and malignancy.[4]

The helminth has the propensity of auto infection and persistence. Extraintestinal infection can involve the lungs, liver, spleen, pancreas, gall bladder, kidneys, thyroid, brain and meninges, skin, mesenteric lymph node, ovaries and skeletal muscles[5], however cases of gastric involvement is very rare. The larvae can invade the stomach by ingestion of respiratory secretions or retrograde migration from small intestine. Eosinophilia is common in Strongyloidiasis, ranging from 25-35% in acute cases and 6-8% in chronic cases. However, in immunosuppressive condition, such as corticosteroid administration, eosinophil counts are lower, and its absence in patients indicates a poor prognosis. Thiobendazole and Ivermectin has been the drug of choice for the treatment of Strongyloidiasis.

Strongyloidiasis is a curable disease and early diagnosis and therapy will reduce the complications and morbidity in immunocompromised patients. We are presenting this case because of the rarity of gastric involvement.

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For journal publications, the 'Format' refers to the manner in which the entire manuscript is organized and presented. There are different types of manuscript formats, as decided by a publishing house/publisher and every journal has a format that is strictly followed. In fact, the format of the manuscript is the first thing being noticed by the editorial team of a journal when a manuscript is received for consideration to be published. Publication of even technically sound manuscripts can be delayed due to their non-adherence to the prescribed format and in certain instances, the manuscript even loses its chance of being accepted and published by a journal. Thus, adherence to the prescribed format is of considerable significance when it comes to preparing a manuscript towards submission to a journal.

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- All contents related to manuscript submission should be in English on a White paper of A4 size with margins of 25mm (1 inch) width on all the four sides.
- Print should be on one-side only with double spacing throughout.
- Pages should be numbered consecutively, beginning with title page.
- Lettering should in Times New Roman with a font size of 12.
- Three paper copies should be submitted to the editor.
- In addition to the paper copies, a digital copy should also be submitted through e-mail or on a compact disc.
- Studies involving human subjects or animals should have received the approval of the institutional ethics committee. A statement about this should be mentioned in the methods of the manuscript.
- Pictures having visible identity of patients should be accompanied by a duly signed patients consent form.

Authorship criteria :

Authorship credit should be based only on substantial contributions

1) to conception and design or acquisition of data or analysis and interpretation of data;
2) drafting the article or revising it critically for important intellectual content; and
3) final approval of the version to be published.

Conditions 1, 2, and 3 must all be met

GUIDELINES FOR ORIGINAL ARTICLE:

Articles of original research are welcome in this category. Articles should not exceed 4000 words. It must include an abstract of 250 words. Minimum of three MesH words to be mentioned at the bottom of the abstract. Upto 50 references may be included in these articles. The manuscript should be prepared as title page, abstract and keywords, introduction, materials and methods, statistical analysis, results, discussion, acknowledgement , references, tables and figures . Each of the above mentioned should begin in a fresh pag .

I. TITLE PAGE: List

(i) title of the manuscript

(ii) the initials followed by the name of each author and highest academic qualification;

(iii) the name of the department(s) and institution(s) to which authors are affiliated;

(iv) the name, address, contact number and email of the corresponding author to whom queries and proofs should be sent.

The authors are strictly advised not to mention their name and affiliation details in any of the subsequent pages other than the Title page since it may interfere with the review process.

II. ABSTRACT AND KEY WORDS: The second page should carry a structured abstract of not more than 250 words with subheadings of

(i) Background and objectives,

(ii) Methods,

(iii) Results and

(iv) Conclusions .

It should be written for the readership of both clinicians and basic investigators and should state the hypothesis or central question of the study or investigation, the study subjects or experimental animals, observational and analytical methods, the main findings, and a final statement of the principal conclusions. Three to six key words using, where possible terms of medical subjects headings list from Index Medicus [MeSH].

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IV. MATERIAL AND METHODS. The material (patients, laboratory tests, experimental animals, etc.) used for making observations must be described along with all other relevant information. The methods used in the study should be described, giving sufficient

information to permit the work to be repeated. If a generally accepted technique has been used, only a reference to that is enough. If, however, such a technique has been modified by the workers, the manner in which this has been done should be clearly stated.

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The relevant statistical methods used for analysis should be briefly explained mentioning the objective of each statistical test in relation to the variables in the reported study that is meaningful. When 'p' value is mentioned the exact number should be mentioned [exception is a highly significant value which may be mentioned as <0.001]. Mention should be made about the predetermined level of 'p' value which will be considered significant. Details of the statistical software used and its version needs mention.

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VI. DISCUSSION. This should be limited to significance of results obtained and what can and what cannot be concluded and why. It should not be a repetition of the findings already given under 'Results'. Results should be discussed in the light of others' work in the field. Speculative and purely theoretical discussion to which results presented are not related will not be accepted.

VII. ACKNOWLEDGEMENTS: Acknowledgement should be brief and made specific for scientific/technical assistance and financial supports in the form of grants/drugs/ equipment only .

VIII. REFERENCES: References should be typed on a separate page after the text and these should be numbered consecutively in the order in which they are first mentioned in the text. In accordance with best practices in scientific writing, latest articles published in relevant area must be referenced. Identify references in text, tables, and legends by Arabic numerals in parentheses.

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Tian D, Araki H, Stahl E, Bergelson J, Kreitman M. Signatureof balancing selection in Arabidopsis. Proc Natl Acad Sci USA. In press 2002.

BOOKS AND OTHER MONOGRAPHS

3. CHAPTER IN A BOOK

Meltzer PS, Kallioniemi A, Trent JM. Chromosome alterations in human solid tumours. In: Vogelstein B, Kinzler KW, editors. The Genetic Basis of Human Cancer. New York: McGraw-Hill. 2002; pp 93-113.

4. CONFERENCE PAPER

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. EuroGP 2002: 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale Ireland.

5. DISSERTATION

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6. ELECTRONIC MATERIAL:

Dengue haemorrhagic fever: diagnosis, treatment, prevention and control. 2nd edition. Geneva : World Health Organization. available at: http://www.who.int/csr/resources/publications/dengue/Denguepublication/en/

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Correctness of the reference list is the entire responsibility of the author (s).

IX. FIGURES AND TABLES

FIGURES.:

(i) Glossy print photographs (in triplicate) are required (usually 10 cm × 8 cm); good contrast is essential for good reproduction.

(ii) Figures (not more than 4) may be submitted in a digital file, preferable in a JPEG (or) TIFF format.

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(iv) All Figures must be numbered and cited in the text.

(v) Legends should be provided for each figure , listed on a separate page.

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TABLES:

(i) Each table should be typed double-spaced on a separate sheet.

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(iv) Explanatory matter should be in a footnote, not in the title. The symbols *,†,‡,§,||,¶,**,††,‡‡ can be used in the table or its foot note..

GUIDELINES FOR REVIEW ARTICLE:

Articles addressing an theme of current interest is welcome in this category. Articles should not exceed 4000 words. The manuscript should be prepared as title page, abstract and keywords,

introduction followed by discussion, acknowledgement, reference , tables and figures . Each of the above mentioned should begin in a fresh page

I. ABSTRACT AND KEYWORDS:

(i) In an unstructured format not more than 250 words.

(iii) It should describe the background and summary of the discussion related to the topic of interest.

(iii) Minimum of three MesH words to be mentioned at the bottom of the abstract. Upto 50 references may be included in these articles.

II. INTRODUCTION: It should commence on separate page and should briefly explain the reason for the review. This should be a brief overview about what is already known on the topic of the article. This should be followed by a statement on the method of review of literature. A systematic explanation of the methods followed to search the literature on the topic of interest is desirable.

III. DISCUSSION: Topic being reviewed in the article should be extensively researched and it should be arranged in a logical manner with relevant subheadings. illustrations , flow charts and tables should be used to explain the text. It should conclude with a brief statement on current opinion on the topic of discussion and future of the same. A hypothesis for future research may also be generated.

Title page, acknowledgement, references , tables and figures should be prepared as per instructions already mentioned under guidelines for original article.

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Properly analyzed cases reflecting important clinical problems that contribute to the understanding of pathogenesis, diagnosis and management of a condition are welcome for this section. Manuscripts discussing more than one case will be given preference. The manuscript should not exceed 750 words with no more than 2 tables/ 3 figures and 10 references. The manuscript should be arranged as title page, abstract, Introduction, description of the case and discussion, acknowledgements, references , tables and figures.

ABSTRACT: It should be no more than 200 words. It should highlight the clinical importance and salient features of the case. 3 Mesh words should be provided.

INTRODUCTION: A brief mention about the background literature related to the case discussed. This should focus on epidemiology and clinical relevance of the case.

DESCRIPTION OF THE CASE AND DISCUSSION: The case should be narrated in a simple and logical manner with important observations shown as tables and figures[the latter two should be kept at the end of the manuscript as described earlier]. Discussion should focus on similar or related case reports published in the global literature and important or unusual features in the case described.

Title page, acknowledgement, references, tables and figures should be prepared similar to instructions already mentioned.

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Thoughtful discussions of current topics are welcome in this category . Should be no more than 500-1000 words, no tables or figures and references to a maximum of 10. The manuscript should be prepared as title page, abstract of 150 words with 3 Mesh terms, text of the manuscript which may be self styled followed by references

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Summary of recent advances in health sciences and education are welcome in this category. Should be no more than 500-1000 words, not more than 2 tables/2figures and no more than 10 references. The manuscript should be prepared as title page, abstract of 150 words with 3 Mesh terms , text of the manuscript which may be self styled followed by references

GUIDELINES FOR IMAGES IN MEDICINE:

Interesting images which is of practical importance are welcome for this section. It should not exceed 300-500 words.Should be prepared as title page, text which briefly explains the details of the image with a short relevant discussion, images which are appropriately labeled and accompanied by legend and references no more than 5. Images should be as per our instructions mentioned under original article. Abstract is not required.

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Correspondence with comments on a recently published journal article or scientific content not related to a journal article [case report, research protocol, original research,etc] is welcome. Word limit is 300 words and 5 references. Abstract is not required. Title page should be as already described.

The submission should be accompanied by

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Three Paper copies of the manuscript in the prescribed format should be submitted to Dr. P.V. Vijayaraghavan, Chief Editor - SRJM & Director (Academic Administration) Sri Ramachandra University, Porur, Chennai - 600 116.

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If hard copies of images are not available then a soft copy is sufficient.

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